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Microbial Health Considerations of Soil Disposal of Domestic Wastewaters
Proceedings of a Conference on May 11-12, 1982
Held at University of Oklahoma, Norman

National Center for Ground Water Research
Norman, OK

Prepared for

Municipal Environmental Research Lab.
Cincinnati, OH

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16. ABSTRACT

The proceedings contain 16 papers from invited investigators recognized for their expertise in the field of microbial health considerations of soil disposal of domestic wastewaters. The topical reviews and recent research papers concerned with current practices in subsoil disposal and land applications of wastewaters, background information on soil and gehydrological considerations, the transport and fate of pathogens in wastewaters, soils, and groundwaters, epidemiologists approaches, and engineering control measures for subsurface disposal and land application of wastewaters were presented before a panel of experts including environmental engineers, virologists, epidemiologists, parisitologists, a soil physicist, a sanitary and public health engineer, and other attendees. The panel members were allowed priority questioning of the speakers during the discussion period following each paper. Following the conference, the panel met to develop recommendations relative to this subject concerning research needs and the approaches that should be taken in future studies. The discussions following each paper and the panel report are included in the proceedings.

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MICROBIAL HEALTH CONSIDERATIONS OF SOIL DISPOSAL OF DOMESTIC WASTEWATERS

Proceedings of a Conference May 11-12, 1982
University of Oklahoma
Norman, Oklahoma

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MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
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U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

PREFACE

In planning the May, 1981 Conference, it was acknowledged that the major objectives, evaluating the current research data and identifying further research needs, could best be achieved by independent senior scientists and engineers who are well recognized for their expertise in the various disciplines and specialties that constitute the subject area. From this point of agreement, a conference format was developed that called for specific topical reviews and recent research papers by invited investigators active in their respective fields. The papers were to be presented before a panel of experts and other attendees. At the conference, panel members were allowed priority questioning of the speakers during the discussion period following each paper. These discussions are included in these Proceedings. The report of the panel is also included without Agency review or editing. We believe that this format provides the Agency and interested readers the most meaningful information with independent reviews and recommendations. The delay in the availability of the printed report is regrettable. However, the information is still current and we trust these Proceedings will be a valuable resource to those having interest and responsibilities in the subject area.

L.W. Canter

E.W. Akin

J.F. Kreissl

J.F. McNabb

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ACKNOWLEDGMENTS

The sponsors of the conference wish to thank each speaker for his presentation and timely submission of a manuscript and the panel members for their peer review and guidance offered to the sponsoring EPA laboratories. Lastly, we thank Lee Huffaker and the staff of the National Center for Ground Water Research, University of Oklahoma, for their tireless efforts in conducting the conference and preparing the Proceedings and Ronald F. Lewis for coordinating the internal peer reviews at the Cincinnati EPA Laboratories and for the final preparation for publication.

TABLE OF CONVERSION
FROM CUSTOMARY TO METRIC SYSTEM

1 acre (ac) = 0.405 hectares (ha)

1 acre-foot (ac-ft) = 1.23 x 10^3 cubic meters (m^3)

1 cubic foot (ft^3) = 28.3 liters (L)

1 cubic foot (ft^3) = 2.83 x 10^{-2} cubic meters (m^3)

Degrees Farenheit (°F) - 32 (5/9) = Degrees Celsius (°C)

1 gallon (gal) = 3.785 liters (L)

1 gallon per day per square foot ($gal/day/ft^2$) = 4.07 x 10^{-2} cubic meters per square meter per day ($m^3/m^2 \cdot d$) .

1 inch = 2.54 centimeters (cm)

1 mile = 1.609 kilometers (km)

1 million gallons per day (MGD) = 3.785 x 10^3 cubic meters per day ($m^3 \cdot d$)

1 minute per inch (min/in) = 0.394 minutes per centimeter (min/cm)

1 ton = 907.2 kilograms (kg)

REPORT OF PANEL
ON
CONFERENCE ON MICROBIAL HEALTH CONSIDERATIONS OF SOIL DISPOSAL OF DOMESTIC WASTEWATERS

This report presents the findings and recommendations of the panel that was formed to participate in the Conference on Microbial Health Considerations of Soil Disposal of Domestic Wastewaters held at the University of Oklahoma on May 11-12, 1981.

The panel reviewed the sixteen state-of-the-art papers presented at the conference, raised questions, and discussed with the speakers the research on which they reported. Following the conference the panel met on May 13, 1981 to develop recommendations relative to microbial health considerations involved with the application of domestic wastewater to soil.

The state-of-the-art papers contain many suggestions for additional research. Although the panel has not listed these, as such, many of the specific research needs expressed by the authors will, of necessity, become part of the multidisciplinary approach recommended in this report.

The following served as members of the Panel:

Don J. D'Alessio, M.D.
James M. Davidson, Ph.D.
Michael B. Gregg, M.D.
Edwin H. Lennette, M.D., Ph.D.
M. Dale Little, Ph.D.
S. M. Morrison, Ph.D.
Wesley O. Pipes, Ph.D.
Russell Rycheck, M.D., Dr.P.H.
Joe A. Salvato, P.E.
Gerard A. Rohlich, Ph.D. - Chairman

INTRODUCTION

The application of wastewaters to land as a means of treatment and disposal is used widely throughout the world. It is estimated that about 30 percent of the year-round dwellings in the United States are served by septic tank or cesspool-soil absorption systems. Land disposal of liquid wastes is also used to some extent by industry and by municipalities. Although, at present, the volume of wastewater disposed to the land by municipalities is a small fraction of the total volume of municipal wastewater available, there is a trend toward increased use of the land treatment process. It is likely that this trend will continue particularly for smaller communities in locations where adequate and suitable land areas are available.

A primary consideration in the treatment and disposal of domestic wastewater, by any of the methods currently in use, is the potential for transmission of infectious diseases. The continued occurrence of waterborne outbreaks of diseases involving surface and ground waters, testifies to the potential for human health problems. From the information available, however, the dimensions of this problem are by no means clear, nor are the mechanisms of occurrence and etiologic agents involved well defined. Epidemology must be refined to relate agent types and numbers and the non-reported subclinical enteric diseases. Information presented at the conference confirms that there is significant under-reporting of outbreaks and often incomplete investigation of those reported.

Control of waterborne diseases results from the use of the multiple barrier concept. Sanitary collection and transport of human waste, wastewater treatment, effluent disinfection, protection of potable water supply sources, treatment of water, well water protection by proper construction of wells, and proper design and construction of water distribution systems are some of the barriers to transmission of waterborne diseases.

It is clear from case studies that infectious agents can be transmitted to ground water supplies as a result of the application of human wastes to the land. Although there are land disposal systems which work without causing apparent public health problems, such systems present a potential for contamination unless properly designed, constructed and operated.

PROGRAM APPROACHES

It is axiomatic that research on environmental problems requires the combined efforts of workers from a variety of disciplines. Although a great deal of valuable information has been and will continue to be provided by individuals working on specific aspects of an environmental problem, the panel agreed that the near-future research programs on land and septic tank disposal should include major support for well-organized multidisciplinary projects. Studies should be planned, reviewed, initiated and the results assembled using the input of soil scientists, sanitary engineers, geologists, statisticians, microbiologists, and epidemiologists. Such projects should be planned and conducted by experienced investigators representing the various disciplines required by the project.

It is also important that appropriate interagency approaches are used so water studies incorporate the talents and contributions from health, food and agriculturally-oriented agencies. Within EPA there is the need for rapid, accurate transmittance of information among laboratories, headquarters offices, field operations, and regional offices. The basic need is to assemble existing data so that it is readily available for use in on-going research projects. Maximum cooperative use of state agencies and the university research communities need to be made. Research projects carried out should be developed with a sensitivity for the ground water problems. Because great geographic differences exist around the country, each project should focus on providing knowledge applicable to many areas. A comprehensive study of the health effects of ground water biological contaminants should be made by the National Academy of Science or a special advisory committee. The study should include an evaluation of the research program and a summary of the state-of-the-art on microbial health considerations of the soil disposal of domestic wastewaters.

The principal focus should be on epidemiologic investigations which consist of field studies to evaluate interactions between microorganisms and chemical substances in the wastewaters and soil, geologic formation, and hydrologic conditions of the disposal area.

Additional laboratory studies will be required in parallel with the field investigations, but it is only the latter which will provide the information required to study the interactions that actually occur and their relative importance.

RESEARCH NEEDS

I. EPIDEMIOLOGY

The panel agreed that increased epidemiologic investigations involving waterborne infections is a first-level priority research need. These studies would supply new information regarding:

- The extent of the current problem - definitions of the numbers and demographic profile of the human population involved.
- The relative importance of the pathogens responsible for outbreaks of waterborne disease(s) transmitted by ground water.
- The mechanisms which coalesce to produce an outbreak include engineering, geological, soil characteristics, and hydrologic factors.
- The extent of microbial transport through soils to ground water as well as in ground water.
- The inoculum size necessary to produce disease and the development of better indicator systems.

There are at least three epidemiological approaches possible -- investigation of waterborne outbreaks, study of endemic (sporadic) cases produced by contaminated water, and study of the infection experience due to on-site exposure of workers at land application systems.

These approaches are listed in order of the panel's judgment of their priority, with better investigation of outbreaks being clearly the most important because it is in many ways the easier approach and most promising as far as producing useful information.

A. Epidemiologic Investigation of Suspected Waterborne Outbreaks (Retrospective)

Investigations of outbreaks should not be limited to ground water sources, at least initially, since useful information may be gathered from surface water outbreaks as well. The research approach will require prior arrangements and cooperation between groups with different capabilities and expertise and will require a mechanism other than investigator-initiated grant proposals.

Certain necessary components are:

1. The creation, between the Environmental Protection Agency (EPA) and the Centers for Disease Control (CDC), of an identified stand-by capacity for rapid response to outbreak notification. For optimum results it will be necessary to begin the medical and environmental epidemiologic investigations simultaneously and as early as possible.

2. Prior agreement with state (and local) health agencies to increase and improve surveillance efforts and a willingness of these agencies to involve CDC and EPA immediately. All state health agencies should be informed of the interest but identification and special arrangements with cooperative states containing high risk areas, if such can be identified, may be more profitable.

3. More comprehensive diagnostic microbiological studies of human cases. Recently there has been noticeable improvement in diagnostic technology for several of the candidate etiologic agents (e.g., Hepatitis A, Norwalk-like agents, Campylobacter fetus, Yersinia enterocolitica, and enteropathogenic E. coli).

4. Similarly, more thorough microbiologic and environmental work on the water source is needed not only to define the relative importance of the various etiologic agents but also to provide information on proper indicators and movement of microorganisms through defined soil types.

Components 3 and 4 may require agreements to contract services on an outbreak basis, with laboratories (university, industry or government) having special capabilities in diagnostic and environmental microbiology, as well as an ability to respond rapidly.

B. Study of Endemic Cases

EPA should consider the need to fund research proposals designed to determine endemic rates of infection/disease believed to be caused by microbial contamination of drinking water, and by malfunctioning septic tank systems. EPA may be able to support CDC financially so coordination between states would be possible. This coordination could result in 6-8 health jurisdiction areas established for surveillance of water-borne diseases.

The rationale behind such studies relates to the knowledge that microbial agents (viruses in particular) can be found in drinking water and wastewaters but their infection/disease burden has not been assessed. Moreover, with the likelihood of increased use of land application and septic tank systems as a method of wastewater disposal in the coming years, a careful microbial monitoring of water supplies and surveillance of human populations should be made.

There is no question that such a project would be expensive to support, and difficult to implement. The project should extend over a 4-6 year time period and be multi-disciplinary in nature. The study should include 1) identification of a proper locale (site with a high risk for having a wastewater disposal problem), 2) contributions from cooperative state and/or local health departments, and 3) infection experience due to on-site exposure of workers at land application systems.

C. Study of Infection Experience at Land Application Systems

Although several epidemiological studies have been reported recently, the impact of on-site exposure to the health of workers exposed to wastewaters at soil disposal sites should be studied by continued, long term, surveillance of such populations.

An outline of a program is given below:

1. Prospective Studies:
   - a. Populations
     - 1) On-site exposed population
     - 2) Controls
       - i) Family
       - ii) Neighborhood
       - iii) Others
   - b. Method
     - 1) On-going monitoring of health
       - i) Identify infectious disease
       - ii) Sub-clinical Infections (microbiological and parasitic workup, serological)
2. Case Control
   - a. Cases of disease or infection identified in (1) above.
   - b. Controls from (1) above.

II. MINIMUM INFECTIVE VIRAL DOSE

Data are required to establish viral dose-response curves which can be used to ascertain exposure levels for risk assessment. Because the portal of entry (route of infection) plays an important role in infections, the minimal infective doses for a disease outbreak should be determined for wastewaters (including aerosalised wastewater) and finished waters.

III. PARASITOLOGICAL PROBLEMS ASSOCIATED WITH LAND DISPOSAL

In considering the possible health effects resulting from the soil disposal of domestic wastewaters on soil, the parasites must be considered separately from other microorganisms. Unlike bacteria and viruses, the resistant stages of parasites are not likely to be transported through soil to ground water unless a significant amount of the wastewater reaches ground water directly through macropores, fissures, etc. The larger size of the resistant stages of parasites (10 to 20 μm for Giardia and Entaboeba histolytica cysts and, generally, >40 μm for helminth eggs) should prevent their passage through most soils. Likewise, parasites from septic tanks and municipal sewage treatment plants are unlikely to pass through soil to ground water.

On the other hand, there is a possibility that parasitic infections could result from exposure at land application sites; the degree to which this could happen is unknown. There is inadequate information available in this country on the types and levels of parasites which occur in domestic wastewaters. Most of the attention has been given to the occurrence of parasites in domestic sludges, but in a few instances where field observations have been made, significant levels of helminths eggs, especially Ascaris and Trichuris, have been detected in secondary-treated effluents from some municipal wastewater plants. While it would be expected that most of the resistant stages of parasites occurring in domestic sewage would be removed from the wastewater during primary treatment, and even more so during secondary treatment, breakdowns in treatment procedures through the overloading of systems, malfunctioning of equipment, etc., could result in the passage of significant numbers of parasites from the plant in the effluent. If such wastewater is then applied to the land, the resistant stages of some parasites, especially the eggs of Ascaris, Trichuris and Toxocara, could be expected to persist for relatively long periods of time under certain conditions. Human infection with these and other parasites might then result from direct contact with the soil, through handling or eating contaminated food crops grown on the land, or by eating animals that had grazed on the land or had eaten crops grown on the land.

The severity of human infections due to parasites that are likely to be associated with the application of wastewater to soils would normally be related to the infective dose. Light infections are generally asymptomatic or subclinical while heavy infections are almost always associated with disease. Consequently, the transmission of these parasites under the circumstances mentioned above is likely to be at low levels and the resulting infections are likely to go undetected unless a surveillance program is in effect. It should be mentioned that there are a few parasites that can produce a serious disease in humans, even for light infections. One example is the ocular disease produced by the larvae of Toxocara canis, the dog round worm. In such infections, even a single larva can produce a serious eye disease. Another example is hydatid disease which can result from the ingestion of a single egg of Echinococcus granulosus or E. multilocularis.

In summary, parasitic infections resulting from the land application of domestic wastewater, if they do occur, are unlikely to occur in outbreaks but as isolated and sporadic cases which are likely to go unnoticed unless an effective surveillance program is in operation.

Priority should be given to determining whether or not wastewaters are likely to be a significant source of resistant stages of parasites. This could be determined by regularly monitoring, over a period of several months to a year, a few selected municipal wastewater plants for the types and levels of resistant stages of parasites present in the effluent wastewater. If significant levels of parasites were found, the next step would be to examine field sites where contaminated wastewater was being applied to the soil in order to determine the concentrations of these parasites in the soil and their survival.

Pilot surveillance programs established to monitor the possible health effects of the land application of wastewaters should include methods for detecting parasitic infections as well as infections due to other types of microorganisms.

IV. SOIL-WATER STUDIES

Unsaturated soil-water conditions prevail much of the time in land application and septic tank systems. This condition may be induced through the effluent application rate or crust formation at the soil-drain interface. Unsaturated conditions are preferred in these systems because of the increased residence time of microorganisms in the soil and opportunity for their inactivation. In order to improve the design of septic tank systems and manage land application systems, greater attention must be given to measurement and use of unsaturated hydraulic characteristics of soils. Site selection should include a surface operated mapping system and absorption test for the proposed septic tank or disposal area. In addition, the rate and composition of water transported through the unsaturated zone must be better quantified for these systems if ground water recharge is to be increased and its contamination prevented. A moisture mapping system could be developed for detecting leaking tank and vault systems.

Because of the heterogeneous nature of field soils, a serious spatial variability condition exists and as a result portions of the soil may conduct significantly larger quantities of water than other portions (in some location the difference may be orders of magnitude). For example, in well structured soils, channels and plane voids produced by roots and worms may be responsible for a major portion of the water flux during temporary saturated or ponded soil-water conditions. The extent to which these situations enhance ground water recharge and transport of pathogens to ground water must be quantified. Although low effluent application rates and soil surface crusting may reduce the phenomenon, the extent of the reduction is not known.

An interdisciplinary group with a working knowledge of saturated and unsaturated flow, as well as solute and particle transport, need to address, quantitatively, the use of soil hydraulic characteristics (saturated and unsaturated) in designing septic tank systems. This information should also be used in siting their location in the environment with respect to surface and subsurface water supplies.

The siting of these facilities should not be based on a predetermined set of distances from water sources, but rather on the behavior of the system in response to specific loading rates, natural rainfall and evapotranspiration, as well as soil properties. Bacterial growth and crust formation in septic tank drain fields control the degree of unsaturation under the drain field; however, this is generally not a controlled variable. It would, therefore, seem desirable to conduct fundamental research on bacterial growth and control, and microbial characteristics, in septic tank systems to establish a predetermined (based on soil hydraulic properties) crust to achieve a desired degree of unsaturation and pathogen inactivation.

A favorable water balance is necessary for the successful operation of a soil absorption system. In addition to wastewater reduction, soil and site characteristics, consideration must be given in the design and construction for control of precipitation entering the system. Control of the water infiltration into the absorption system, and the amount of soil evaporation, and the surface vegetation transpiration taking place at various times of the year, can determine the hydraulic loading on the absorption system and the transport of pathogens into and possibly through the unsaturated zone below the distribution system laterals.

A study is needed to determine the percent runoff expected for various rainfall intensities, infiltration rates, and different soil types from grass covered subsurface absorption systems (fields, beds, mounds, also sand filters, evaporation - transpiration systems) with a uniform topsoil and ground slopes of ¼ in., ½ in., 1 in., and 2 in. per foot. This information is required in order to design and minimize the hydraulic loading on the absorption system (including sand filter and evapotranspiration systems) and the transport of pathogens through the soil system.

V. MONITORING AND SURVEILLANCE

Intensive monitoring and surveillance is necessary to establish baseline conditions, and to determine if land disposal systems have been properly designed and are being operated properly. Baseline studies should be made on the microbial populations in "undisturbed" ground water in areas having different geological characteristics. These studies should include not only microorganisms of health significance, but also those of significance in the transformation of organic components of wastewaters. A major question in a monitoring program of soil disposal systems, or in reported outbreaks, is the value of a bacterial indicator system as indicative of the presence of viral agents. A comprehensive review of this subject is needed.

The monitoring program should be designed to provide statistically valid spatial (where to monitor), and chronological (when to monitor) data, to demonstrate the effectiveness of the particular land disposal operation under investigation.

Monitoring and surveillance programs should include measurement of a conservative chemical substance, in parallel with the microbial measurements, so that die-away and/or inactivation of the microorganisms can be estimated.

"The work described in this paper was not funded by the U.S. Environmental Protection Agency. The contents do not necessarily reflect the views of the Agency and no official endorsement should be inferred."

INITIAL SESSION

OPENING REMARKS

by

L.W. Canter

Professor of Civil Engineering and Environmental Science
Co-Director, National Center for Ground Water Research
University of Oklahoma
Norman, Oklahoma

On behalf of the University of Oklahoma and the National Center for Ground Water Research, welcome to the opening session of the "Conference on Microbial Health Considerations of Soil Disposal of Domestic Wastewaters". This Conference is sponsored by three research laboratories of the U.S. Environmental Protection Agency, namely, the Health Effects Research Laboratory and the Municipal Environmental Research Laboratory, both located in Cincinnati, Ohio; and the Robert S. Kerr Environmental Research Laboratory located in Ada, Oklahoma. The Conference host is the National Center for Ground Water Research (NCGWR). The U.S. Environmental Protection Agency (EPA) established the NCGWR in September, 1979, as a consortium of the University of Oklahoma, Oklahoma State University, and Rice University. The primary focus of the NCGWR is to address the four major isues identified by the EPA as problem areas that persist in ground water protection. These include transport and fate of pollutants, characterization of the rate-determining factors in the subsurface environment, development of methods for ground water quality assessment and protection, and information transfer.

All technical sessions of the Conference will be held in this room, the Forum Room, of the Oklahoma Center for Continuing Education. This

Center was established in 1961 and is located on the University of Oklahoma campus. If you have any questions relating to the Center or its programs, or need assistance in terms of travel or other arrangements, you can contact any member of the NCGWR staff present at this Conference.

I want to commend each of you for choosing to attend the Conference despite the fact that this first day follows the Sunday celebration of Mother's Day. I realize that this can create difficulties in terms of travel planning, and I do hope that the Conference will meet the needs which you have felt were sufficient to justify your attendance.

The objective of this Conference on Microbial Health Considerations of Soil Disposal of Domestic Wastewaters is to provide a forum which will lead to the development of prioritized health-related research needs associated with the application of domestic wastes to land through the use of septic tank systems and land application systems. The Conference was basically planned and developed by a four-person committee consisting of Dr. Elmer Akin from the Health Effects Research Laboratory, Mr. James Kreissl from the Municipal Environmental Research Laboratory, Mr. James McNabb from the Robert S. Kerr Environmental Research Laboratory, and myself.

To achieve the stated objective of the Conference, a tailored program format and design has been developed. The Conference will consist of the presentation of technical information by a series of invited speakers. The speakers were identified by the planning committee and they have been asked to prepare state-of-the-art papers on specific subjects. These papers form the basis for the Conference Program distributed to each of you at the registration desk.

In addition to the invited speakers, .the planning committee identified a select panel of nationally-recognized experts in several technical fields related to the Confernce. These panelists are charged with the responsibility of reviewing papers, listening to the verbal presentations, and asking the speakers questions. Following the Conference the panel will prepare a report which will contain recommendations relating to the health-related research needs associated with the application of domestic wastewaters to land. The need for carefully prioritizing research needs is becoming increasingly important since Federal budgetary planning indicates limited resources for achieving a variety of environmentally-related research needs. To formulate the recommended research needs, the panel will be meeting on several occasions at this Conference and will have follow-up communications. Specific panel members and their substantive area are as follows:

Dr. Gerard Rohlich (Panel Chairman) -- Environmental Engineering -- Department of Civil Engineering, University of Texas, Austin, Texas.

Dr. James Davidson -- Soil Physicist -- Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida.

Dr. Donn d'Alessio -- Virology -- Department of Preventive Medicine, University of Wisconsin, Madison, Wisconsin.

Dr. Michael Gregg -- Epidemiology -- Center for Disease Control, U.S. Department of Health and Human Services, Atlanta, Georgia.

Dr. Edwin Lennette -- Virology -- Department of Health Services, State of California, Berkeley, California.

Dr. Dale Little -- Parisitology -- School of Public Health and Tropical Medicine, Tulane University, New Orleans, Louisiana.

Dr. S. M. Morrison -- Microbiology -- Department of Microbiology, Colorado State University, Fort Collins, Colorado.

Dr. Wesley Pipes -- Engineering-Microbiology -- Department of Biological Sciences, Drexel University, Philadelphia, Pennsylvania.

Dr. Russell Rycheck -- Epidemiology -- Department of Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania.

Mr. Joseph Salvato -- Sanitary and Public Health Engineer -- Joseph A. Salvato Consulting Company, Troy, New York.

Each of you has been provided with a brochure describing the technical program of the Conference. The technical program consists of five major parts preceeded by a keynote address. The keynote address on microbial health considerations will be given by Dr. Jay Lehr, Executive Director, National Water Well Association, Worthington, Ohio. The five topical areas for paper presentations include current practices, background information on soil and geohydrological considerations, transport and fate, epidemiological approaches, and engineering control measures. Two papers will be presented on current practices associated with the use of septic tank systems and land application systems. Mr. James Kreissl will discuss sub-surface disposal via septic tank systems, and Dr. Curtis Harlin will summarize land application systems.

Background information on the movement of pollutants through the soil to ground water will be presented by Dr. J.C. Lance and Dr. Wayne Pettyjohn. Dr. Lance will address soil considerations and Dr. Pettyjohn the geohydrological considerations related to this subject.

Five papers will be presented on the transport and fate of pollutional constituents resulting from the soil disposal of domestic wastewaters. Parasites will be addressed by Dr. Paul Fitzgerald, and bacterial pathogens in soil by Dr. Richard Weaver. Bacterial pathogens in ground water will be described by Dr. Charles Hagedorn. Viruses in soil and ground water will be addressed by Dr. Mark Sobsey and Dr. James Vaughn, respectively.

Epidemiological approaches for evaluation of the soil disposal of domestic wastewaters will be presented in a series of five papers beginning with virus occurrence in ground water by Dr. Charles Gerba. Outbreak data compiled by the Center for Disease Control will be summarized by Dr. Rickey Wilson. Dr. Victor Cabelli will address endemic transmission. The concept of minimum infectious dose will be presented by Dr. Elmer Akin, and risk evaluation techniques will be described by Dr. Robert Cooper.

The final portion of the technical program will consist of two papers related to engineering design and control measures for septic tank systems and land application systems. Dr. William Boyle will address septic tank systems, and Dr. Charles Sorber will summarize control measures for land application systems.

You are encouraged to ask questions following each paper presentation. Time has been allocated for questions and responses. I would like to request that you state your name and affiliation prior to asking your question since we are recording these sessions and the transcripts will become a part of the Conference Proceedings. The Proceedings will be published following receipt of the panel report and completion of the transcript development. Each of you as registrants for the Conference will receive a copy of the Proceedings.

At this time I want to particularly acknowledge the planning and development work by Ms. Le Huffaker, Technical Writer-Editor, National Center for Ground Water Research. Ms. Huffaker works at the University of Oklahoma and has spent countless hours working on Conference arrangements and contacting the panelists and speakers. She has also been involved in planning the use of the facilities here at the Oklahoma Center for Continuing Education, as well as planning the social activities relating to this Conference. In addition, I want to acknowledge the assistance of Dr. Marcia Bates, Associate Professor of Civil Engineering from Oklahoma State University. Dr. Bates has worked with us in the general planning for the Conference, and she will be involved in future activities associated with the Conference Proceedings. In addition to these two individuals, I would also like to acknowledge the general assistance of the staff of the National Center for Ground Water Research in their participation in the planning and development of the Conference.

In closing, let me again welcome you to this Conference on Microbial Health Considerations of Soil Disposal of Domestic Wastewaters. The program has been carefully planned, and I personally am looking forward to excellent technical presentations on the state-of-the-art in this important substantive area. On behalf of the National Center for Ground Water Research let me invite you to participate in the Conference and get acquainted through both formal and informal sessions. I am looking forward to a lively and successful Conference. Thank you very much.

"The work described in this paper was not funded by the U.S. Environmental Protection Agency. The contents do not necessarily reflect the views of the Agency and no official endorsement should be inferred."

KEYNOTE ADDRESS

The Rain to the River and the Sewage to the Soil

by

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In spite of the great Roman Aqueducts, purposeful quality control of water supply and wastewater disposal is quite recent in origin. It began with the growth of cities emerging from the Industrial Revolution in the 19th Century. City slums resulted, and soon, as Gordon Maskew Fair of Harvard put it in his early text on wastewater disposal, "The Apocalyptic Horsemen of Pestilence and Death Rode with Rein Unchecked."

Storm drains which had been around for centuries were not allowed to be used for waste discharge until late in the 19th Century. But when waste piles became too great a problem, it was allowed and wells and rivers became polluted.

Late in the 19th Century a lawyer, engineer and nobleman by the name of Sir Edwin Chadwick prescribed the solution we discuss here today, "The rain to the river and the sewage to the soil."

Although my name has become synonomous with "ground water" to many over the last several years because of my role as Executive Director of the National Water Well Association, I am well aware that ground water is only one part of the total environment. Through my role as a member of the National Drinking Water Advisory Council, I am aware of the full extent and nature of our water resources in this country and the problems they face.

I have been and am an advocate of ground water, but only to the extent that ground water be given its fair share of consideration when decisions concerning this Nation's water resources are made. Half of this Nation's population depends upon ground water as a source of drinking water, and half of the Nation is dependent upon surface water.

The point that we often fail to consider is that ground water and surface water are not discrete, separate entities, but interconnected parts of the overall hydrologic cycle. Decisions made concerning one will have an affect on the other sooner or later. We saw this illustrated very clearly several years ago on Long Island where a decision to discharge wastes from sewage treatment plants into the ocean, primarily to prevent ground water contamination, only resulted in greater ground water problems, as well as off-shore pollution.

That decision, like many, was made in ignorance and supported by convenience. Today we are paying the price for our lack of knowledge and absence of forethought. In the future, we hope to do better. However, we are still faced with the knowledge that our wastes have to go someplace. Under the circumstances, we have some hard choices to make. These choices concern beneficial use, risk analysis and priorities associated with our knowledge of and appreciation for the interrelationship of air, land, and water in our total environment. We must decide what wastes will be tolerated in the environment and in what amounts. We must determine under what circumstances it is better for us to deposit our waste products on land, in the air, or in the water. We must recognize that in the disposal of wastes, convenience, expediency, and low costs must be considered, but also may be shortcuts to environmental deterioration and endangerment of human lives.

However, when environmental decisions are made with full knowledge of their subsequent ecological, health, legal, social, and economic effects, we will bring order to our actions, and environmental control regulations can then be designed to have both direction and purpose.

To make such decisions requires a great deal of knowledge. Such knowledge may already exist. In other cases it will have to be developed through further research. The problem is ascertaining what is known and then deciding what to do next with limited resources.

That is why this conference is extremely important.

It is important because the ultimate product of this conference will be prioritized recommendations for future research associated with the application of domestic wastes to land. To make recommendations in this area means that the panel must decide whether current practices of land application of domestic wastewaters present a health hazard or need to be improved. They must decide whether we have enough information to even decide whether there is a health hazard. They also must decide whether we possess sufficient knowledge of the factors which determine the fate of pathogens during land disposal to even design effective systems for protecting the public from microbial disease. The implications of the panel's decisions will be far-reaching. The panel which will make these decisions has been carefully selected for its scientific knowledge, experience, and most importantly, openmindedness. I believe that the panel's recommendations will carry a great deal of weight because of the panelists' reputations for fairness, their scientific integrity, and because their varied backgrounds will give them the proper perspective of the total problem.

Granted, EPA is not bound to their recommendations. However, I must assume that the recommendations will have a great influence on future EPA research, as well as on regulatory measures and waste disposal practices.

I have been encouraged in recent years by EPA's efforts to seek the opinions and advice of individuals from industry, environmental groups, other government agencies, and the scientific community. EPA has not always done so in the past, a shortcoming which I have pointed out to them editorially numerous times. Recent experiences make me believe that EPA is truly seeking outside guidance in developing strategies to managing our water resources.

The decisions made here will be important to many in the research community. To support EPA's priorities, ORD's research must be responsive to EPA's regulatory and enforcement needs. ORD's first research priority is to address those pollutants which are judged to be the greatest health risk. Because toxics are currently considered to be our most pressing problem, resources for toxics research has increased substantially.

Similarly, the budget of the Solid and Hazardous Waste Research Program, also once modest, has begun to rise dramatically in response to increased awareness of problems associated with hazardous waste disposal. This upward trend will most likely continue until problems raised by the Love Canal incident and other abandoned waste disposal sites are solved.

In contrast to the above programs, the Water Quality Research Program, which traditionally had a large budget, has consistently experienced budget decreases over the last several years. Moreover, the focus of the program has shifted from research on the more traditional pollutants, including microbial pathogens, to toxic substances. The majority of the budget decrease has been in water control technology research.

If the trend to decrease funding in the areas dealing with microbial hazards and water control technology is to be reversed, a strong case of need in these areas must be made to have any chance of competing for EPA research funds.

EPA must be financially accountable. Under the new administration, EPA must learn to spend its money wisely and well. It has experienced budget cuts both in money and personnel and will experience more. Because EPA will not be able to fund all proposed research, research considered to be irrelevant or low-priority probably will not be funded.

The cost-effectiveness of various waste-management alternatives will be an important consideration when environmental decisions are made. Already, there have been major cutbacks in the construction-grants program for sewage treatment facilities. Municipalities will continue the move toward land application of wastes because of its comparative low costs.

There will be a continued reexamination of existing regulations and criteria and a close scrutiny of new regulations. Are they technically necessary to protect the public and the environment from pollution or are they the result of a bureaucratic rule-making machine gone wild?

The results of your deliberations during the course of this conference can make a substantial contribution to the Nation's quest for knowledge and in the shaping of decisions which will guide future activity in this subject area. I wish you well as you undertake the earnest debate that will shape your sessions, as you attempt to resolve honest differences of opinion that are sure to arise, and as you labor to reach the common concensus which undergirds our democratic process.

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CURRENT PRACTICES

CURRENT PRACTICES - SUBSURFACE DISPOSAL

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ABSTRACT

Review of the onsite wastewater system technology literature reveals a dearth of data on the occurrence and fate of pathogens. Similarly, in light of recent advances in the field of soil physics, the microbiological literature on the fate of pathogens in soils reveals that existing information is often less than helpful in an analysis of the potential transmission of these varieties. Consequently, an assessment of the potential pathogenic groundwater problems accruing from onsite wastewater treatment systems cannot presently be more than qualitative until better data are provided for the fate of organisms in the all-important unsaturated soil zone below the disposal system. Although the problems of the initial startup period for conventional onsite system designs can be qualitatively described, there is clearly a need to develop quantifying data through the joint efforts of microbiologists and soil scientists in order to provide the engineers and sanitarians with the health-related justification to improve onsite system designs.

Additional areas where data are sparse include the occurrence of pathogens in septic tank pumpings and their fate and effects through the handling, treatment and disposal of these residuals. Also, the quantification of the potential health risks due to the failure of soil absorption systems by surfacing is relatively undetermined.

Introduction

Since the earliest days of recorded history, man has demonstrated a continuing and growing desire to separate himself from his waste products. In early times this desire was based primarily on the offensive nature of wastes, but as knowledge of disease sources and transmission progressed, the desire proved to be well-founded as a means of minimizing the incidence of many, but not all, diseases.

Armed with new confidence that improved sanitation techniques could control many formerly unavoidable diseases, the simplicity and effectiveness of the septic tank-soil disposal concept which both carried wastes safely from the home and disposal of them below the surrounding soil could be clearly appreciated. The application of this concept was limited by two factors extant prior to World War II. Since the concept was considered viable only where no sewers were available and it required continuous water supply availability on demand which, in turn required electricity, the general lack of cheap electrical service in rural areas limited adoption. Also, the overall demographic trend of decreasing rural populations leaving the farm for jobs in the industrializing urban centers contributed to the modest growth in septic tank-subsoil disposal technology.

In the period immediately following World War II factors such as low-cost mortgage availability, sudden population booms, inadequate supply of housing, inexpensive and improved transportation, and relative prosperity resulted in housing demands both in areas surrounding urban centers and in more remote communities now served by modern electrical networks as a result of the rural electrification programs begun some years earlier. The resulting condition was a tremendous increase in the number of homes served by septic tank-subsurface disposal systems. The state and local authorities were ill-prepared to deal with these problems since they had minimal information on which to base the siting, design, construction, and maintenance of these facilities. The resulting concern for a major breakdown in the level of sanitation due to wholesale failures of these systems precipitated a major study by the Public Health Service to investigate the proper functioning of unsewered individual household wastewater sanitation systems. The results of this major research program served to significantly upgrade the state of knowledge on the siting, design, and maintenance of onsite wastewater disposal systems.

Although much of the information generated by these studies was incorporated into the ensuing document that has become a classic in this field, i.e., the Manual of Septic Tank Practice (1), the overall text of that document was not completely reflective of the Public Health Service studies. The Manual of Septic Tank Practice (MSTP) did provide a national uniform code basis, which was wholly or partly incorporated by most state health departments into their codes. Although many clearly undesirable onsite system practices were eliminated by this procedure, the MSTP was by no means perfect; for example, it promoted the desirability of sewers as the only "permanent solution to wastewater disposal." However, the popular demand for further onsite wastewater system research was diminished for a substantial period as a result of the popular misconception that the MSTP contained "all the answers."

By the 1970's, a series of events were underway which caused renewed interest in onsite disposal systems. Major contributors were inflation and the initial satiation of large cities' demands for wastewater treatment facilities. This combination moved the plight of the smaller communities to the forefront of eligibility for federal funds. However, costs for traditional "preferred" solutions, i.e., conventional sewers and treatment methods, employed almost exclusively in larger cities were found to be prohibitive for these smaller communities, even with large federal and state subsidies. The first reaction to small community demands for solutions to wastewater contamination problems was to provide research and development information on lower cost onsite alternatives and new sewering technologies which could reduce the cost of abating problems caused by failing existing systems in smaller communities. Much of the improved onsite technology will be discussed later in these proceedings by Professor Boyle.

An additional aspect of this work has been devoted to better understanding of how conventional systems work, and why they fail to perform in the manner originally conceived, i.e., disposal which keeps the liquid waste below the ground surface and has minimal impact on the quality of underlying groundwater. Although some onsite system failures will undoubtedly always occur, the great majority are due to improper site evaluation, inadequate design, poor construction techniques, and little or no maintenance. In short, onsite wastewater treatment and disposal technologies are technically feasible, but have traditionally been improperly implemented. Despite this, it is not uncommon to find data which indicate systems have performed successfully anywhere from 20 to 40 years.

In order to satisfy the stated purpose of this discussion, i.e., description of current practices in onsite subsurface disposal, it was necessary to review an extensive body of microbiological information on the occurrence of various organisms, their characteristics and response to environmental conditions and, most importantly, removal data from field or laboratory simulations of field treatment and disposal systems. Upon completion of this review, in light of onsite technology advances, the need for this conference became quite clear. The prevailing literature does not provide the necessary data upon which public health decisions can be based with any reasonable certainty.

Source Characteristics

The role of conventional septic tank-soil absorption systems (ST-SAS's) in controlling the spread of pathogenic microorganisms and the fate of these organisms within the confines of this technology are difficult to define due to significant variations in state and local codes, in enforcement of those codes, and in the functioning of these systems in different soils. Figure 1 depicts a complete ST-SAS within its surroundings. In essence, wastewaters generated in the household are conveyed via the building drain and sewer to the septic tank, where settling of heavier solids and flotation of lighter materials takes place. From the septic tank the partially treated wastewater flows to a subsurface disposal structure where the wastewater may infiltrate into the soil for further purification prior to incorporation into the groundwater below the site.

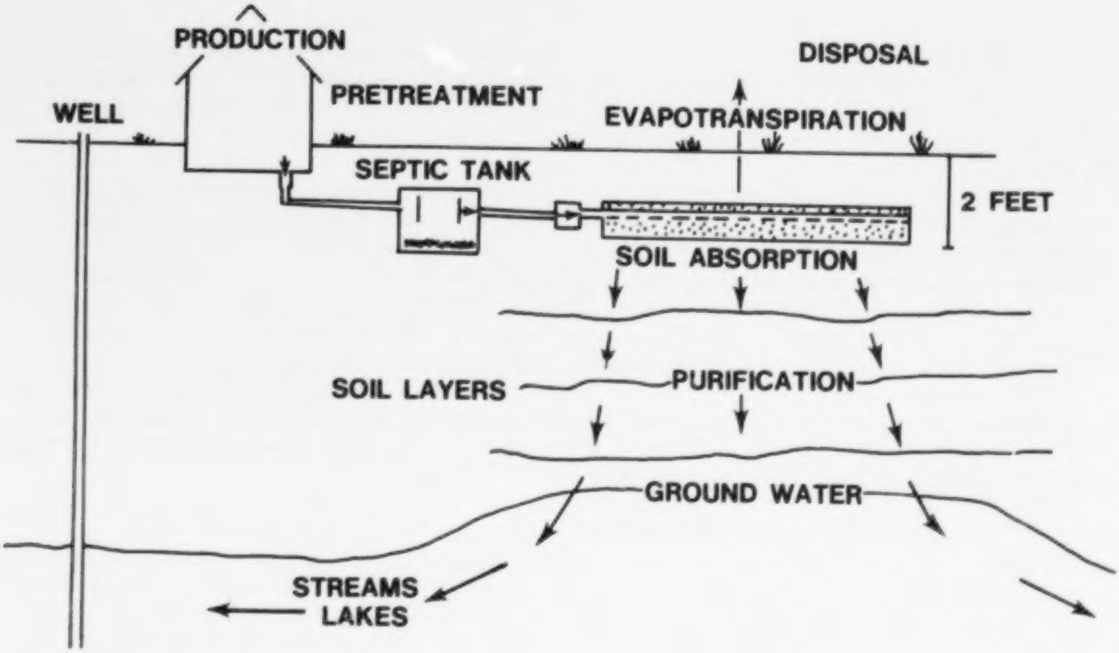


Figure 1. Soil Disposal of Septic Tank Effluent

As a first step to defining current practice, without regard for its fallacies or shortcomings, one needs to characterize the wastewater from the household served. Several investigators have recently analyzed household wastewaters in terms of flow, chemical and physical parameters, and traditional microbiological measurements, the most complete study being that of the University of Wisconsin (2). Unfortunately, there are relatively few data on specific pathogens in household wastewaters. However, one distinct difference between household and municipal wastewater should be noted, i.e., the presence of specific pathogens is less probable in household wastewaters, but when present their concentration will normally be higher. Put in simpler terms, a particular endemic pathogen will usually appear in a municipal wastewaters in low numbers, but will likely appear in a specific household wastewater only when one or more members of that household are actively shedding. The pathogen will likely be far less diluted than in the municipal system unless either a high percentage of the community is infected or the pathogen sources are not limited to human fecal discharges. However, once introduced to a septic tank, it may persist for some time.

Studies of several rural households for the pathogens Salmonella spp., Pseudomonas aeruginosa and Staphylococcus aureus were positive in 2 of 11, 10 of 10, and 4 of 10 families' wastewaters, respectively (3). Indicator bacteria, as measured by fecal coliform and fecal streptococci tests were ubiquitious. Similarly, Green (4) reported that only one of several septic tank effluents was found to yield a positive test for virus (140 PFU/l), that being identified as a poliovirus 3 (Po-3). Recent California studies of composting toilets and graywater (non-toilet wastewater) systems isolated one Salmonella organism from 26 samples of 12 toilets, while 2 of 20 samples from 11 toilets yielded reovirus, and 26 samples from 12 toilets yielded one Trichuris trichiura ova (5). All compost toilet leachates analyzed were positive for nematode larvae and/or living flagellate and ciliate protozoa.

Therefore, the first questions to be answered are those pertaining to what organisms are present in household wastewaters, in what numbers and how do they respond to the environment of the septic tank? To the first question, in addition to Table 1, the literature sources report the potential presence of parasites Ascaris, Hymenolepis, Trichuris, Toxacara, Taenia, Enterobius, Ancylostoma, and other hookworm and nematode species as well as numerous bacteria and virus species. Of course, the number of these organisms in the household wastewater would vary widely from zero to very large numbers per liter when contributors become infected. Such quantitative numbers are somewhat scarce, but could be estimated by assuming a weight of feces excreted times the number of organisms per unit weight of feces from infected individuals, diluted by the daily wastewater flow from the dwelling.

Septic Tanks

A cutaway view of a single-compartment septic tank is shown in Figure 2.

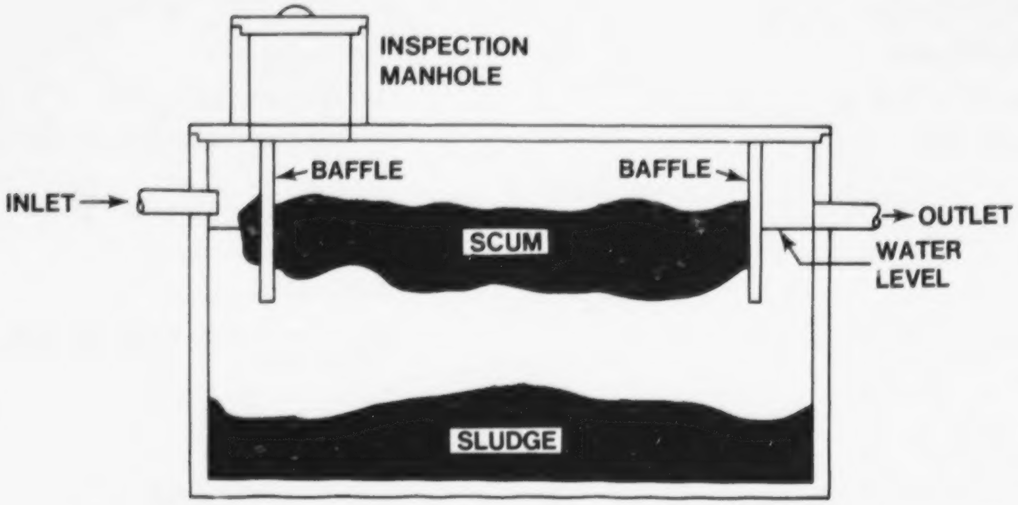


Figure 2. Single Compartment Septic Tank

TABLE 1. PATHOGENIC AND OPPORTUNISTIC MICROBES IN HUMAN WASTEWATER (5)

Microorganism	Disease Caused
Bacteria	
Salmonella spp.	Typhoid, Paratyphoid, Gastroenteritis
Shigella	Bacillary Dysentery
Yersinia	Gastroenteritis
Mycobacterium	Tuberculosis
Leptospira	Leptospirosis (Weils Disease)
Pathogenic Coliform	Gastroenteritis, Urinary Infections
Pseudomonas	Respiratory and Burn Infections, Diarrhea
Klebsiella	Pneumonia, Bronchitis
Serratia	Respiratory and Urinary Infection, Summer Diarrhea, Urinary Infection
Viruses	
Polioviruses	Poliomyelitis
Hepatitis Type A	Infectious Hepatitis
Echoviruses	Respiratory Disease, Diarrhea, Fever
Coxsackie viruses	Respiratory Disease, Fever
Norwalk-like Agents	Gastroenteritis
Rotaviruses	Gastroenteritis
Adenoviruses	Respiratory Disease and Eye Infections
Parasites	
Entamoeba histolytica	Amoebic Dysentery
Giardia lamblia	Gastroenteritis

Although multiple compartment tanks and improved inlet and outlet structures have been demonstrated to improve performance of septic tanks, the majority of the approximately 20 million septic tanks in use in the United States are similar to the type shown. Septic tanks have been installed over a period of decades under a variety of codes which have tended to increase the required liquid volume from old levels of about 1.9 m^3 (500 gallons) to 3.8 m^3 (1,000 gallons) or more in present codes. In the tank, heavier solids tend to settle, while lighter solids tend to float forming the sludge and scum layers, respectively. Typical solids removals vary with tank design, ambient temperature and nature of the incoming wastewater which is a function of habits and diet of the household, appliances, climate, and other factors. Well functioning septic tanks will usually produce effluent suspended solids between 40 and 70 mg/l, but actual removal efficiencies are difficult to compute due to the varying type and frequency of wastewater generation events entering the tank. Also, the tank will often reach an active digestion stage during warmer periods, interfering with solids removal mechanisms, particularly with single-compartment tanks.

The fate of the various organisms in wastewater in septic tanks is depicted in Figure 3.

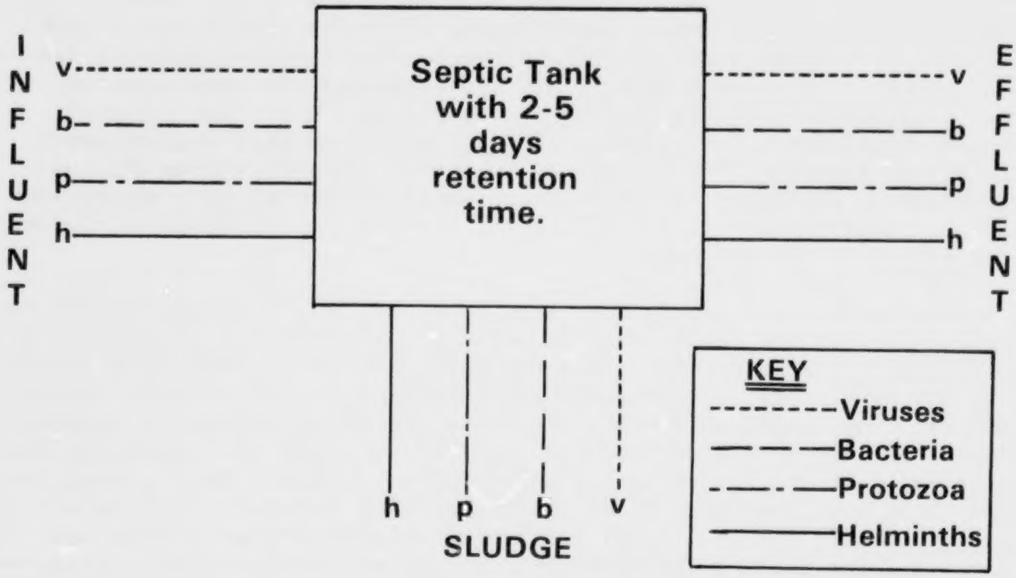


Figure 3. Fate of Pathogens in a Septic Tank

There is a dearth of information on specific organism removal in septic tanks. However, the tendency of organisms to become associated with solids is well recognized Also, the nominal hydraulic detention time of septic tanks can be estimated based on average flow per capita of 190 l/day(50 gal/day)(2),the 1.9 and

3.8 cu m (500 and 1,000 gallon) sizes noted above, and an average family size of four persons. These assumptions would yield a nominal detention time of 2.5 to 5 days. The World Bank Report (6) notes that removals of pathogens in septic tanks are a function of detention time and organism reaction to the oxygen deficient conditions in the tank. Removals for all pathogens are given as 0 to 2 logs (0 to 99%). Howard and Lloyd (7) report 2 to 3 logs of removal of *Vibrio cholerae* in a two-stage tank receiving only toilet wastes with minimal flush volumes. Also, *V. cholerae* die-off under anaerobic conditions at 25 C was reported to be about 0.5 log/day. The World Bank Report indicates that *Leptospira spp*. normally survive only a few days in wastewater; *Salmonella spp*. removals approximate suspended solids removal although large or multiple-compartment tanks may approach 3-log removal (99.9%); *Shigella spp*. compete poorly with other organisms in septic tanks; *Ascaris* ova were removed at nearly the same efficiency (82%) as suspended solids in a 1.5 m^3 (400 gallon) septic tank treating blackwater with a nominal detention time of 20 days; only 65 percent of the hookworm ova were removed under the same conditions as *Ascaris* ova; settling rates of *Schistosoma* ova were shown sufficient to obtain at least 73 percent removal in two days; *Taenia* ova had been shown to settle slower than other parasite ova; *Trichuris* ova were 98 percent removed in a cylinder and 56 percent removed in primary clarifiers at two hours nominal detention time; *Entamoeba histolytica* cysts were found to have a Stokes settling velocity of 0.007 to 0.11 meters/hr; *Ancylostoma* were found to be 60 percent removed in a municipal septic tank and 98 percent removed by three Imhoff tanks in series; and a septic tank with three-day nominal detention time removed 99.4 percent of *Ascaris* ova with no effect from anaerobiasis. Some investigators (8) have reported minimal removals of virus by primary sedimentation. Green (4) prepared suspensions from feces containing poliovirus and found that shaking was sufficient to free 50 to 89 percent of the associated virus from the suspended solids. Subsequent attempts to estimate the amount of readsorption in a septic tank yielded 18 and 45 percent readsorption to solids with one hour of mildly-agitated contact.

In reviewing these widely scattered data, the only conclusion which can be reached is that the septic tank is unlikely to remove any organism, completely, and that septic tank effluent must be considered capable of transmitting any disease whose pathogenic agent appeared in the influent or raw wastewater. Some support for this conclusion has been provided by epidemiologists. During the period from 1970 to 1974, at least ten waterborne disease outbreaks in the United States have implicated septic tank systems (9-11). Five of these outbreaks were infectious hepatitis; two were shigellosis (*Shigella sonnei*); two were acute gastroenteritis of unknown origin; one was typhoid fever (Salmonella typhosa); and one was cholera (*Vibrio cholerae*). It should be noted that this number of outbreaks is less than 10 percent of the total number of outbreaks occurring during this period and that ST-SAS's serve between 20 and 30 percent of the U.S. population. Also, in all confirmed ST-SAS causations, recommended separation distances were met or exceeded.

Soil Absorption Systems

The above conclusion provides reinforcement to the key role played by the subsurface soil disposal system as the primary barrier to passage of pathogenic microorganisms into the environment. To understand the difficulty of describing current practices in this crucial area, Table 2 is presented.

TABLE 2. VARIABILITY OF STATE CODES

Setbacks (to wells, surface water)	11 -92 m	(35-300 ft)
Trench Spacing	1.8- 3.0 m	(6- 10 ft)
Trench Cover	0 - 0.3 m	(0- 12 in)
Min. Perc Rate	Yes-No	
Max. Perc Rate	12 -47 min/cm	(30-120 min/in)
Trench Width	0.3- 0.9 m	(12- 36 in)
Sizing	Soils-Perc Rate	

In addition to the varying code requirements described in this table, several other phenomena must be discussed before any analysis can be attempted. The unsaturated zone of soil below the bottom of the disposal system is the single most important factor in preventing transmission of pathogens. This zone provides physical straining, aeration/drying, adsorption potential and other physical and chemical factors which can maximize both removal and inactivation of pathogenic organisms. What is not shown in Table 2 is the widely varying and often inadequate site evaluation requirements of the state and local codes. Often these shortcomings minimize the effectiveness of the unsaturated zone from the initial moment of use, providing increased potential for pathogen transport to the groundwater or surface where the potential for human contact becomes more imminent and conditions are less suitable for removal.

In analyzing the performance of subsurface SAS's, the method of size determination and liquid distribution must be reviewed. Most states require the use of the percolation test both for determination of soil suitability and SAS sizing. The percolation test is depicted in Figure 4. The physical significance of this test has never been established, but its variability has (12)(13). Given the fact that most existing codes are based to some degree on the MSTP (1), the design sizes of that document are given in Table 3. The variability of a well-performed percolation test has been shown to be anywhere from 20 to 90 percent (12). Therefore, it could be assumed that field or SAS sizing could be in error by at least these percentages for a well-performed test, and possibly much more when the test is poorly performed in fine textured soils.

With a standard trench disposal system the following description is pertinent. In the initial few months of operation, a conventional system with gravity distribution and 10 cm (4-inch) pipe with multiple holes at or near

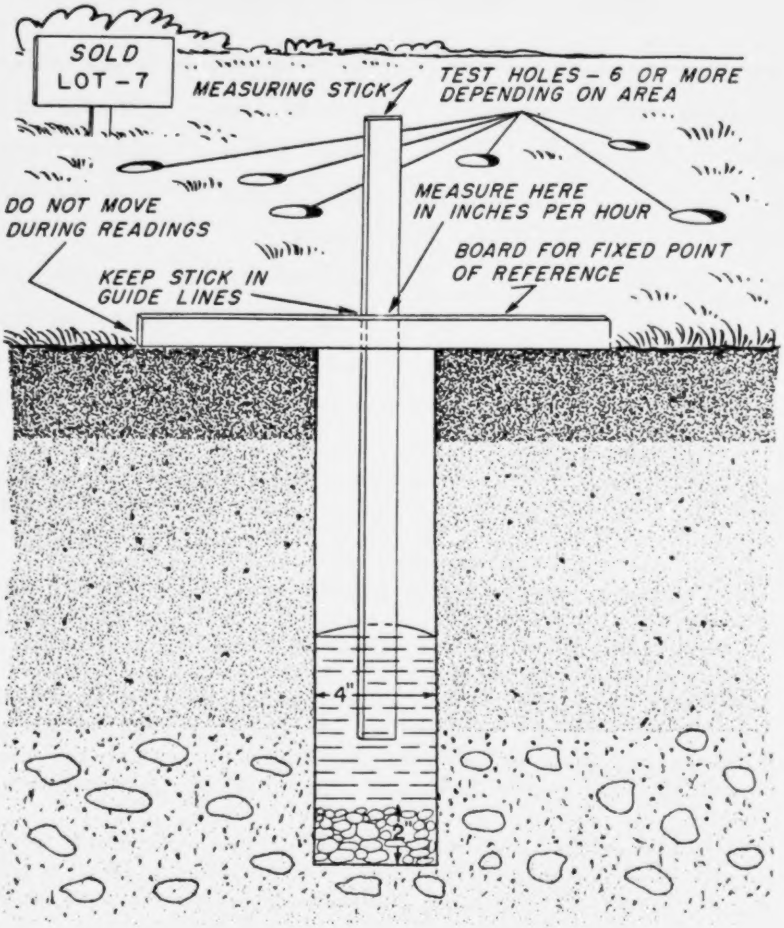

SOLD
LOT - 7
MEASURING STICK
TEST HOLES - 6 OR MORE
DEPENDING ON AREA
DO NOT MOVE
DURING READINGS
MEASURE HERE
IN INCHES PER HOUR
BOARD FOR FIXED POINT
OF REFERENCE
KEEP STICK IN
GUIDE LINES
4"
2"

Figure 4. Typical Percolation Test

TABLE 3. ABSORPTION-AREA REQUIREMENTS FOR INDIVIDUAL RESIDENCES (1)

Percolation Rate (min/in)†	Bottom Area (sq ft/BR)*	Percolation Rate (min/in)†	Bottom Area (sq ft/BR)*
1 or less	70	10	165
2	85	15	190
3	100	30	250
4	115	45	300
5	125	60	330

† 1 min/in = 0.394 min/cm

* Required bottom area per bedroom (BR); 1 sq ft = 0.093 m^3

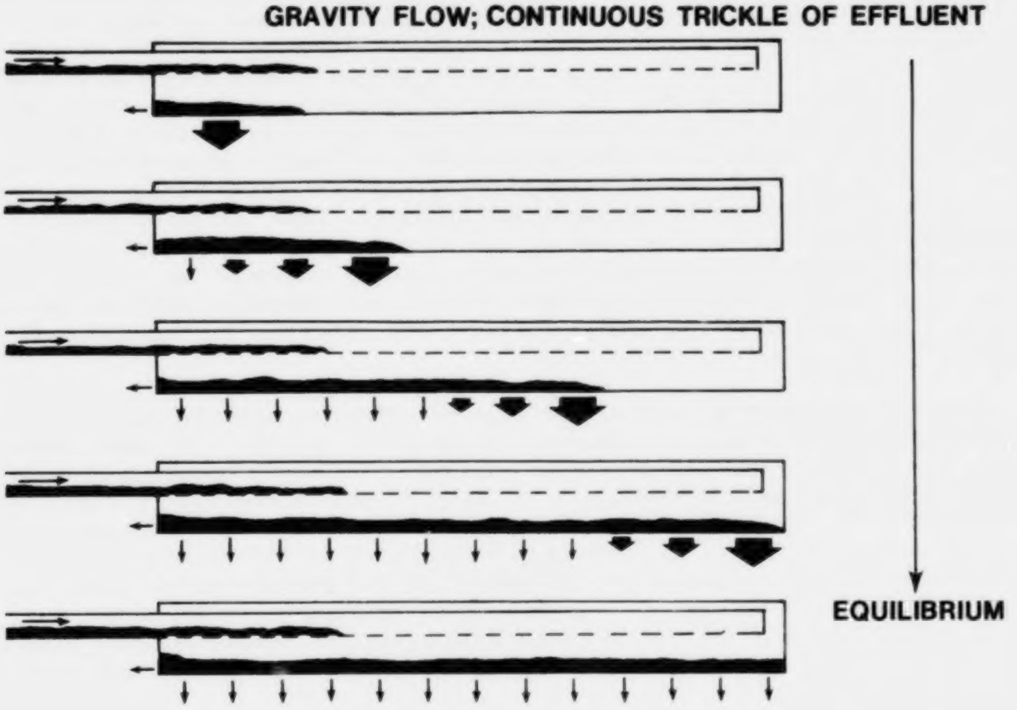

GRAVITY FLOW; CONTINUOUS TRICKLE OF EFFLUENT
EQUILIBRIUM

Figure 5. Distribution and Effects in Conventional Soil Absorption System

its bottom or drain tile with spaces between tiles will distribute septic tank effluent in the manner shown in Figure 5. (One variation which may provide some difference is the serial distribution system primarily used in areas with significant slopes.) In the normal situation, the relatively small septic tank effluent volumes introduced into these large pipes in response to wastewater generating events in the house are transmitted to the initial portion of the trench rather than being equally distributed across the entire disposal field. This results in the liquid being loaded almost exclusively on a very small portion of the trench, creating a localized overloading. For example, if the loaded area were one-tenth of the entire trench, the local loading would be ten times the design loading. This results in a greatly accelerated rate of clogging development and a greatly increased impetus to drive pollutants and microorganisms deeper into the unsaturated zone owing to increased localized velocities and reduced opportunities for drying and aeration during the period prior to crust formation. With time, this phenomenon spreads along the trench moving the localized overloaded zone along the lead edge of the progressing crusted area. Eventually (exact completion times of the process are not known, but six months have generally been used for sandy soils), the crusting covers the entire length of the trench. Assuming that the trench bottom is level, the effluent can potentially spread evenly over the entire surface. Further assuming that the trench is properly sized, the infiltration rate should be equal to or greater than the hydraulic loading rate. This condition is conducive to extremely effective removal of microorganisms. If, as is often the case, the infiltration rate of the crust or the trench bottom area is inadequate to transmit the hydraulic load, effluent ponds in the trench, moving the crusted zone up the sides. If the total infiltration surface under crusted conditions is insufficient to transmit the liquid, surfacing and a surface failure may result. Increased hydraulic head on bottom areas will not appreciably increase infiltration rates in the trench bottom (14).

Assuming the above scenario, the fate of pathogens through the unsaturated zone must be considered in distinct modes or phases: (1) newly operational, (2) crusted, and (3) surfacing failure. The newly operational mode is quite perplexing with conventional or traditional technology as described above because of the danger it could represent. Although new techniques for distribution, as described by Professor Boyle later in this conference, are able to minimize this problem, an assessment of the problem is all that can be made for traditional systems. Such an assessment is very important to this discussion as much of the literature on travel of microorganisms through soils fails to make these distinctions or to realistically test field conditions.

When liquid is applied to a new SAS in any soil, it will infiltrate the soil at a specific rate. That rate will change over a period of time, however, as a crust develops in the vicinity of the trench-soil interface. This is due to the fact that although all soil pores are initially available for carrying liquid and larger pores preferentially carry most of it, these pores begin to be closed off with time, and the smaller pores begin to carry more of the liquid because the larger pores become unavailable due to their inability to draw the liquid through the crust. In each soil this results in different patterns because of different patterns of natural pores and other voids or channels due to earthworms, roots, etc. The longer detention times associated with flow through tortuous small soil pores provides ideal conditions for pathogen removal and inactivation.

Most studies of removal by unsaturated soil have been performed on sandy soils for the simple reason that these are easily and reasonably well reproduced in the laboratory, while more structured soils have rarely been properly studied because of the need to use undisturbed cores of sufficient size for such studies. Sand and silt loam columns were employed (2-4) for virus and bacteria removals in several comprehensive studies in Wisconsin. Virus removals of 5.9 logs were found in 37 cm of 60-cm medium sand columns dosed once per day at 5 cm/day (1.25 gal/day sq ft), but removal was reduced to 2.9 logs through the entire column at 10 times that rate of application. Operation at 6 to 8 C at 5 cm/day daily dose had no adverse effect. However, high rates of loading, e.g., 50 cm/day did result in increased virus penetration at lower temperatures. All experiments demonstrated the value of lower hydraulic loadings in retaining virus and the fact that any dose which exceeds the void volume of the uncrusted or new soil in the unsaturated zone could potentially cause some virus penetration to the saturated zone, i.e., groundwater. Although some movement of retained virus was noted, this movement was not great and was accompanied by inactivation with time. Inactivation was temperature dependent in that at 20 C retained virus was inactivated at 18 percent per day, while at 6 to 8 C, inactivation of 1.1 percent per day was found. Inactivation of poliovirus 1 (Po-1) in septic tank effluent was found to be 29 percent per day at room temperature (20-25 C), while at 6-8 C, it was less than 10 percent per day. Inactivation was generally slower in soil columns.

Parallel studies of bacteria removal reinforced the conclusions of the virus study that new or uncrusted soils must be loaded at low rates of application to prevent the passage of organisms through larger pores which provide minimal opportunities for retention. As a crusting develops with time at the soil-trench interface, passage of organisms becomes minimal because of the loss of large pores for transmission. Experiments designed to simultaneously evaluate two soil types (loamy sand and silt loam), two temperatures (25 and 5 C), and two loading regimes (5 and 10 cm/day for sand and continuous ponding and 1 cm/day for silt loam) were conducted (2). Fecal coliforms were removed by as little as one log during the first three months of sand loading, but removals increased to more than three logs as crusting developed after about six months of operation at 25 C and 10 cm/day loading. Fecal streptococci also penetrated the 60 cm columns and 3 of 19 samples for Pseudomonas aeruginosa (1.4 x 10^3/100 ml added) were also positive. Neither of the latter two tests were positive at the 5 cm/day sand loading at the same temperature. Neither column was found to pass Staphylococcus aureus (10^2/100 ml added). The 5 cm/day loading performed with even better removal when the loading was divided into 1/3 doses at three times/day, illustrating the benefits of multiple dosing vs. once per day dosing at the same daily loading. At 5 C, both loadings quickly produced a ponded condition. Although both columns completely removed S. aureus and nearly all of P. aerugenosa (5% to 10% positive samples), fecal indicators (particularly fecal coliform) were always present at low temperature operation. With the silt loam columns, ponding developed at the 1 cm/day dosing rate in one month, thus eliminating passage of any of the fecal bacteria. One other silt loam column was found to have several large pores or channels which resulted in wholesale passage of all organisms tested, except S. aureus. Reducing the loading from 1 cm/day to 3 mm/day caused the liquid to penetrate the pedons of the soil and eliminated the passage of fecal indicators within 30 days, although P. aeruginosa required 90 days for elimination.

Other investigators have reported information on the travel and/or survival of pathogens in unsaturated soil. Kristiansen (15) reported nearly four logs of fecal coliform reduction in sand which had a clogging layer within 10 cm of the soil-trench interface, while 75 cm failed to remove more than one log in slightly clogged sand. Brown, et al (16), used three undisturbed soils to determine travel of fecal coliforms below the SAS trenches. A sandy loam loaded at 8.2 cm/day (2 gal/day/ft^2) yielded positive fecal coliform samples 5 percent of the time 1.2 meters below the trench during the first year of operation (mostly during initial startup), while second year positives were 2 to 3 percent. All samples after the second year were negative. In the sandy clay loaded at 3.3 cm/day (0.8 gal/day/ft^2) large pores, due to natural cracks or root channels, caused high counts below the trenches during the early days of operation, but fecal coliform counts returned to normal as crusting developed. With the clay soil loaded at 1.6 cm/day (0.4 gal/day/ft^2), travel of fecal coliforms was negligible. In parallel studies with coliphage f2, two of the 52 samples taken from 1.2 m below the trenches in the clay soil tested positive over a one-year period. These two samples that showed 4 logs of removal were taken a few days after large coliphage doses were added to the dry (cracked) clay soil. Robeck et al. (17) obtained 3 to 4 logs of removal of Po-1 through rather uniform sand of 0.25 mm effective size in 0.88 m (1.25 ft) of travel at 82 to 163 cm/day (20 to 40 gal/day/ft^2) loading (once per day feeding). Cram (18) used a relatively uniform sand (e.s. = 0.53 mm) and once per day dosing at 9.5 cm/day (2.33 gal/day/sq ft) to determine removals of parasites from settled wastewater. It was noted that 0.3 m (1 ft) of sand was able to remove 3 logs of the 8 logs of Ascaris eggs applied with the first dose, and no eggs appeared in the effluent after four days. It was further noted that 0.61 m (2 ft) of sand removed all Ascaris and Ancylostoma eggs and Entamoeba histolytica cysts applied through seven days of operation. Ascaris and Ancylostoma eggs were removed by the 0.61 m columns at 12.2 and 16.2 cm/day (3 and 4 gal/day/ft^2).

Numerous other references are available, but after the tests are reviewed, it often becomes clear that the test conditions are either not representative of actual field contions or important data were not recorded. To illustrate this requires introduction of the concept of "contact time" in the unsaturated zone of the soil. Contact time is derived from the actual rate of travel of liquid through a column of unsaturated soil. Quantification of this phenomenon has only been accomplished in recent years and is by no means complete even at this time. Basically, the concept involves the knowledge of moisture retention curves and hydraulic conductivity curves for specific soil types and knowledge of flow characteristics in each. Both of these curves are shown in Figures 6 and 7 (19). Because of its relative uniformity of pore sizes, sand is the simplest soil to use in explaining the concept of contact time. From Figure 6 one can quickly note how a sand drains after all the pores have been filled with water. If, for example, one knows the average moisture content of a sandy soil and the nominal application rate, an average velocity may be computed by the following equation:

$$v' = v/\theta \tag{1}$$

where: v' = average velocity (cm/day)
v = nominal application rate (cm/day)
θ = water-filled porosity (cm^3/cm^3)

In sand with a $v = 5$ cm/day and $\theta = 0.09$, $v' = 56$ cm/day and contact time in each foot (30 cm) of unsaturated soil would be 30/56 days or 13 hours.

The above approach works well with sands since a piston flow exists, i.e., water initially present is pushed out by applied water in a relatively well defined manner since pore distribution is rather uniform. Similarly, coarser sandy loams or loamy sands can be reasonably well described by equation 1 in terms of contact times. However, more structured or fine-textured soils (types 3 and 4 of Figure 7) cannot be analyzed in this manner due to the occurrence of macropores, i.e., channels and planar voids caused by roots, worms, etc., through which most liquid flow occurs in the absence of crusting. In real soils this problem is quite apparent. As an example, this technique is applied to a fine soil with a large vertical pore running the entire length of the unsaturated zone. If this soil was accepting a nominal loading of 3 cm/day and moisture content was determined to be 46 percent by volume, the equation yields $v' = 3/0.46 = 6.5$ cm/day or a contact time of 4.6 days/ft. However, instead of replacing existing water, the new liquid is actually bypassing as much as 95 percent of it, yielding an actual average velocity of $3/0.02 = 150$ cm/day or a contact time of 4.8 hours (20). In order to overcome this short-circuiting, extremely low loadings must be employed with pathogen-laden septic tank effluents to maximize contact time in the unsaturated zone or development of a crust must occur. Both of these techniques increase contact time by making larger pores ineffective in the transmission of septic tank effluent to the groundwater. Examples of the effect of both techniques are shown in Figure 8. The conditions shown in the figure were quantified in chemical engineering terms to show the relative amount of short circuiting by expressing time in units of theoretical detention time based on total pore volume.

The contact time concept is consistent with observations of other reviewers (21) that pathogen removal passage through soil is due to both mechanical straining and adsorption processes. Maximizing contact time improves the effectiveness of both mechanisms. Also, increased contact time is directly related to soil dryness and both increased time and reduced moisture provide greater opportunities for inactivation of pathogens. If a necessary contact time for pathogen removal could be established by concerted efforts of microbiologists, soil scientists, and engineers, some rationality of design could be established for ST-SAS's to replace the "black art" status

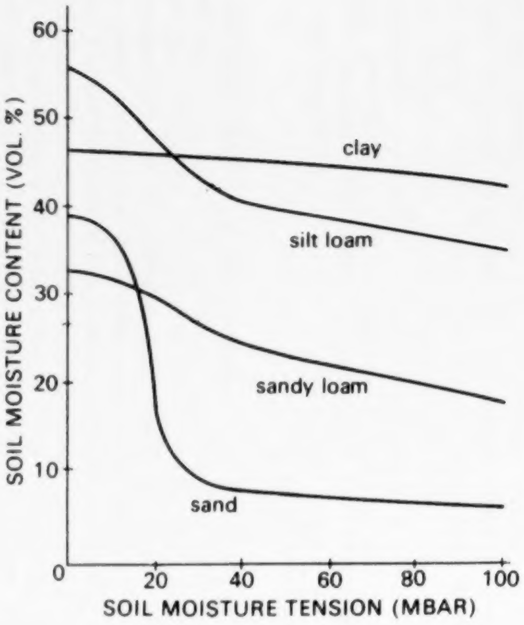


Figure 6. Moisture Retention Curves

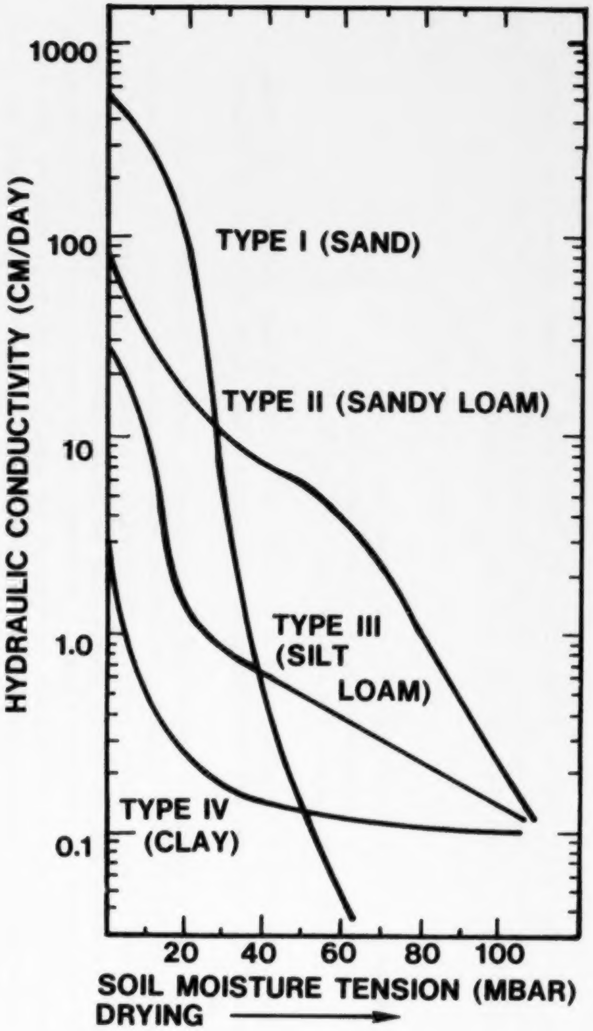
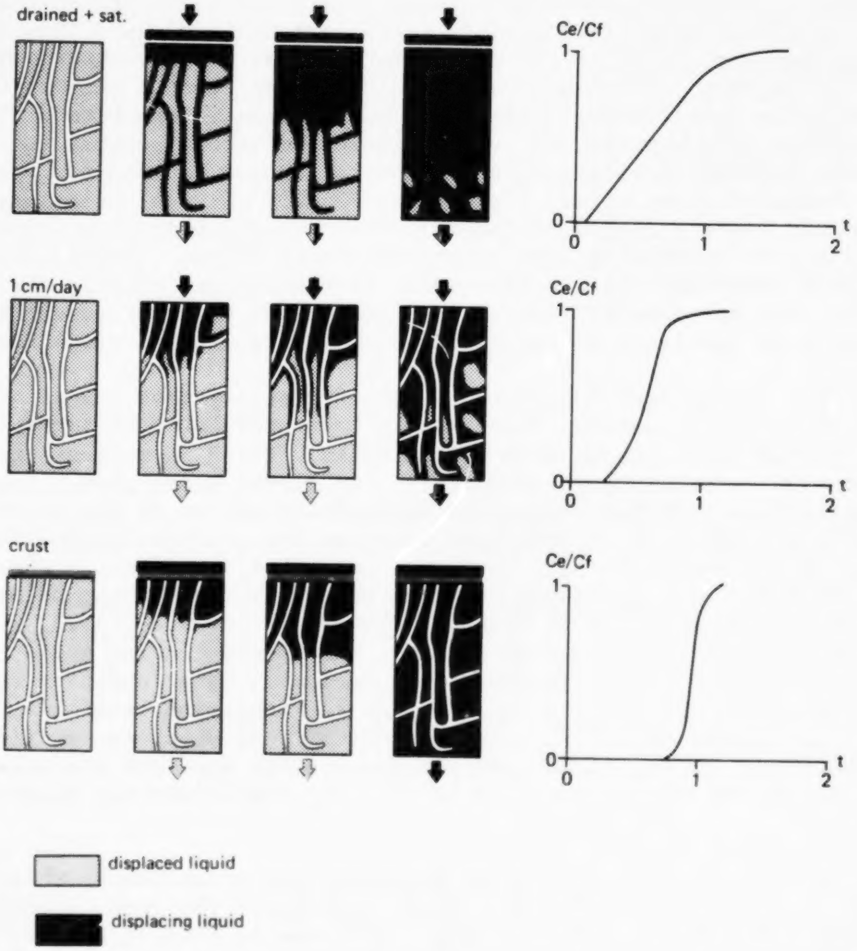


Figure 7. Hydraulic Conductivity Curves


drained + sat.
Ce/Cf
1
0
t
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1
2
1 cm/day
Ce/Cf
1
0
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Ce/Cf
1
0
t
0
1
2
displaced liquid
displacing liquid

Figure 8. Flow Regimes and Breakthrough Curves for a Pedal Soil Under Different Loading Conditions (t = time; C_f = concentration in feed; and C_e = concentration in effluent)

which has long been operative in this field. If, for example, it were determined that a contact time of two days would suffice for removal of all pathogens in the unsaturated zone, designs could be flexible in light of local site constraints rather than restricted by rigid separation distances, percolation tests, etc.

Another aspect of the contact time and other soil physics concepts described briefly herein is that the various literature citations on travel of pathogens in soils, other than certain well-characterized sands, has little meaning and may contribute to the widely varying and even contradictory nature of the state of the art. For example, studies relating removal to particle sizes of soil may only be valid for sands where piston flow is exhibited. In finer soils with high clay content, macropores could completely reverse the reported relationship between clay content and organism removal because the smaller pores are bypassed under actual conditions. Purely chemical relationships such as pH and ionic strength are not affected by soil hydraulics, but their importance may be diminished when short-circuiting exists. Indeed, it appears that the most important measure of removal is the contact time in the unsaturated zone, and any removal figure cited is useless to other researchers without thorough knowledge of the soil and the hydraulics of flow through it.

Two other public health aspects of conventional systems should also be described in this discussion. These are failures resulting in surfacing and the residuals periodically pumped from septic tanks. Based on present state codes, two types of failures are possible. In states which permit field vents, septic tank effluent could have uninhibited access to the surface, resulting in introduction of pathogens to areas where direct access is possible. In states where no field vents are permitted, at least in the initial stages of failure, pathogen laden effluent must pass through approximately one foot of soil cover prior to reaching the ground surface. Also, if a level site and level construction can be assumed, the upward loading would be equally distributed over the entire trench surface. This condition should provide some degree of pathogen removal. As the condition continues, the potential for channeling would increase if sufficient head were available between the house and the field, and the failure may approach the situation described for field vent systems. Both of these conditions are depicted in Figure 9.

Periodically, as scum and sludge accumulations reach levels which could impair the performance of the septic tank, pumping of the tank contents must be performed. The pumped liquid, sludge, and scum is usually called "septage". Several investigators have noted the fact that a wide variety of pathogens survive long periods in septic tanks and other sludges. A study by Noland et al.(22), determined the presence of *Salmonella spp*. and *Pseudomonas aeruginosa* in septage along with parasites *Ascaris*, *Trichuris*, and nematodes. Wolman (23) reported the presence of eggs of *Ascaris*, *Ancylostoma*, and other hookworms in septage. The World Bank (6) cites *Salmonella typhi* survival of 166 days in a cesspool. Fiege et al.(24) reported *Pseudomonas aeruginosa* in all four septages tested and *Salmonella spp*. in one of four. The World Bank (6) noted septage data that indicated two logs of removal for *Schistosoma* eggs in 12 days, one to two year survival of *Trichuris* eggs,

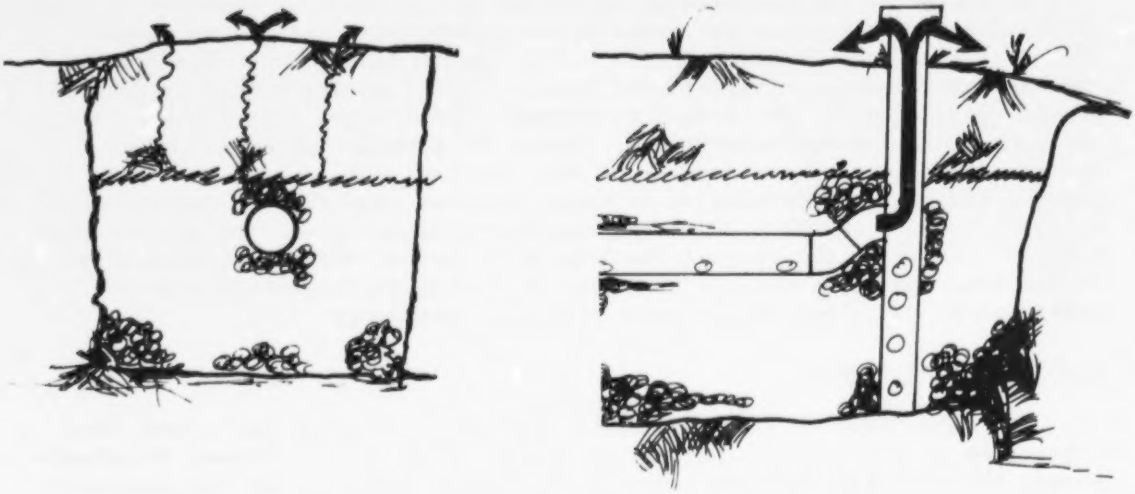

Figure 9. Failure Modes of Two Soil Absorption System Designs

relatively short survival of *Entamoeba hystolytica* cysts, *Ancylostoma* eggs present in a density of 28 eggs/gram of dry solids, and *Ascaris* survival of greater than one year.

The significance of these and other data sources must be viewed in the light that any pathogen introduced to a septic tank can be found in the septage if that introduction occurred within its die-off period prior to pumping. Therefore, the handling and disposal of septage represents a potentially hazardous situation. The disposal of septage to the land has been discussed in several forms and is outside the subject of this discussion. However, epidemiological studies of the disease incidences of septage pumpers would appear to be a subject of potentially fruitful investigation, since this industry is often uncontrolled and handling practices vary widely.

Of the few studies on septage treatment which have included pathogenic investigations, two have involved liming of septage (22)(24). One (22) reported that liming to pH 12.5 reduced *Salmonella spp*. and *Pseudomonas aeruginosa* to undetectable limits and reduced fecal and total coliforms and fecal streptococci by 5, 5, and 3 logs, respectively. However, parasitic eggs were still present and viable. The other (24) showed reduction of both the above bacterial pathogens to undetectable levels by liming septage to pH 10.4 to 12.3. The University of Wisconsin study (2) included treatment of septage by formaldehyde at pH 10 and glutaraldehyde at ambient pH. Both techniques reduced *Pseudomonas aeruginosa* counts below detectable levels at aldehyde concentrations of 500 mg/l or more. Reductions of 3 to 4 logs of fecal indicators also occurred with the 500 mg/l dose of glutaraldehyde, but only about 1 log of Po-1 reduction was found. At a 1,000 mg/l glutaraldehyde dose, fecal indicators were removed by greater than four logs, and Po-1 was removed by about three logs in three hours of contact. All tests were performed at 20 C.

In reviewing the information presented herein there is evidence to quantitatively support a case for problems associated with traditional septic tank soil absorption systems from a public health standpoint. The reasons for the shortcomings in traditional onsite technology are primarily related to misapplication of the technology through poor design, poor construction, and poor operation and maintenance. These are problems which are being overcome through research studies of the last ten years, which have led to greatly increased understanding of these problems, and through communication of these results to state and local regulatory agencies and the general population. However, the basic technology deficiencies which have been identified have spawned studies of alternative onsite systems which will be described by Professor Boyle later in these proceedings.

Health-Related Issues

The major health-related concerns with traditional ST-SAS's have been identified as the initial operational period which poses a threat to groundwater, the periodic problems relating to septage pumping, and the potential problem of system failure and surfacing of wastewater due to natural clogging or improper design or construction practices. All three of these modes of pathogen transmission have been identified as causes of disease outbreaks. The problem of groundwater pollution is the most prevalent, and is intimately tied to the flow of water through unsaturated soils. Uncrusted or fresh soil is capable of infiltrating far higher hydraulic loadings than conventional design criteria, generally described by the MSTP (1), anticipate. Although field studies have shown that the loadings of Table 3 are reasonable in practice at equilibrium conditions (2)(19), these conditions do not exist in newly constructed systems. Due to the poor design of the distribution system and the random generation of household wastewaters, serious localized overloading of the soil occurs. This condition, shown in Figure 5, results in flows many times the design flow and provides the impetus for very deep penetration of pathogens below the trench bottom. Although this condition may be transient, i.e., not usually more than six months in duration, a potential microbiological hazard to groundwater exists. By way of an example, it could be assumed that a four-person household with three bedrooms would require anywhere from about 23 m^2 (250 ft^2) of trench bottom area in a coarse sand to about 232 m^2 (1,000 ft^2) in a relatively fine soil (see Table 3). With the sand, a loading of ten times the design level would easily be accepted by the soil, but would theoretically drive pathogens deeper into the soil. The data of Robeck et al. (17) indicate that this loading would provide at least three logs of removal of poliovirus in 38 cm, while Green and Cliver (25) showed three logs of removal of Po-1 in 60 cm with similar sands and rates. Therefore, if the required vertical separation between the trench bottom and the groundwater were greater than 60 cm (see Table 1), three logs of poliovirus removal would be assumed possible. However, if an infected individual of the household were to shed 100 grams of feces containing 10^6 plaque-forming units (PFU)/gram, dilution in 0.76 cubic meters (average flow for a family of four) would yield an average wastewater content of about 1.3×10^5 PFU/liter. With adhesion to setteable and flotable solids, it is reasonable to assume close to one log of removal of virus in the septic tank. Therefore, if greater than four logs of virus were applied to the trench in the above overloaded manner, approximately one log of virus could potentially reach the groundwater if there were no more than two feet (60 cm) below the trench. Similarly, pathogenic bacteria could penetrate a minimum-depth unsaturated zone, as witnessed by the *Pseudomonas aeruginosa* penetration of 60 cm sand columns cited above (2) (3). Based on the limited data available for parasites, it would appear unlikely that these organisms could penetrate even 60 cm of overloaded sand.

If a finer soil were chosen for the above example and extensive macropore development were extant, the results might be more severe. It would seem plausible that the same distribution shortcoming which caused overloading of 10 percent of the sand trench could cause even greater overloading of the much longer fine soil trench and an overloading of as much as 30 to 40 times the design loading rate. This situation could be quite serious from the standpoint of purification in that the loading would exceed the saturated capacity of the soil in that portion of the trench, resulting in saturated flow through macropores, extremely short contact times, and potentially high numbers of organisms traveling large vertical distances. Although the flow would tend to equalize over most of the trench in clayey soils, macropores may cause significant groundwater contamination if those macropores are continuous through the unsaturated zone. If the example of the macropore effect on contact time used earlier were operative, only 9.6 hours of contact would be available in 60 cm of soil above the groundwater. Although this contact time would appear to be about the same as that of the overloaded sand above, some soil structures with macropores could permit even more pronounced short-circuiting. The experience of Brown et al. (16), in finding coliphage f2 only in the cracked clay soil, verifies the effects of macropores. Potentially, even the larger parasites might penetrate unsaturated soils under such conditions.

The problem of surfacing soil absorption systems has been used by numerous small communities to justify sewers on the basis of imminent health hazards. Although some public health concerns clearly exist and some remedial action is obviously required, there would appear to be a need to evaluate this failure mode as it relates to soil type, cover thickness, venting and stages of failure progression with regard to pathogen movement.

The septage pumper is exposed to a potentially high pathogenic insult during the course of his activity, even in comparison to sewage treatment plant workers. The disease history of these individuals may provide insight into the need for and mode of institutional control to ensure not only their safety, but that of the homeowner and anyone exposed to the septage during its subsequent processing. Also, studies of the fate of pathogens in septage treatment systems are incomplete at this time.

Finally, it would appear that there is a significant need to measure pathogens throughout the onsite treatment and disposal sequence to establish the base conditions and to provide a public health basis for modification of the site evaluation, design, construction, and operation of onsite wastewater treatment and disposal systems. Although many modifications and alternative designs are being developed, the basis for these new approaches has been mostly hydraulic, i.e., to overcome site constraints to disposal. Without sufficient health-related data to demonstrate improved public health aspects, these modifications are resisted by governmental agencies and potential beneficiaries alike because of incremental construction cost increases, even though long-term savings may be inherent. Health-related data should result in more acceptance of alternative systems which could result in greater protection for rural populations.

Conclusions

1. The transmission of pathogens to groundwater is primarily controlled by the conditions in the unsaturated soil located below the disposal system and above the water table.

2. Most existing data on pathogen removal by soils are of limited use because of a lack of sufficient data on the soils and the flow regimes during the period of testing.

3. There is a need for cooperative efforts between microbiologists and soil physicists to properly characterize and measure the ability of different soils to remove pathogens under various conditions.

4. The transmission of pathogens by means of effluent surfacing and mishandling of septage is less insidious than groundwater contamination by otherwise functioning systems.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

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CURRENT PRACTICES - SUBSURFACE DISPOSAL: JAMES KREISSL

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: Rohlich

I have one question. To what extent have there been determinations of the hydraulic characteristics of septic tanks since you continually hear about 2 to 3 days retention?

A: Kreissl

The theoretical detention time of a septic tank prior to accumulation of scum and sludge is generally 3 to 5 days. Typically, discrete wastewater generation events are less than 5 percent of the total tank volume. Therefore, the long detention time tends to obfuscate the poor hydraulic design of the septic tank. To my knowledge, no dye studies have been performed. However, studies of tank suspended solids removal efficiency done by USPHS and the Wisconsin small scale waste management staff indicated the benefits of compartmentalization. There is a real advantage in the multiple compartment tank because it would cut down any potential short-circuiting without increasing overall tank volume requirements.

Q: Rohlich

Well, I don't want to take the time to argue with you on that point, but even in the design of gravity oil-water separators, for example, and other similar units, the problem is that actual flow-through time is often much less than the theoretical detention time.

A: Kreissl

Absolutely correct, but the point that I am making is that since the theoretical detention time is so long, the actual detention time would still be in excess of that for primary clarifiers. However, your point is well taken in that improved septic tank hydraulics has not been fully explored.

Q: Morrison

Jim, would you like to comment on the fact that a great number of these systems are in second homes, e.g., recreational homes, in which your hydraulic loading runs through an annual cycle of peaks and valleys. Is there any hope of getting a system which will adjust to this kind of difficulty pattern.

A: Kreissl

Quite obviously, I think, because of its non-mechanical nature, the septic tank-soil absorption system is probably a good approach. From the standpoint of the soil, there are significant benefits to a long period without use. Soil infiltration capacity becomes regenerated during periods of non-use. From the tank standpoint, because of its non-mechanical nature, it is least affected by that type of wastewater generation pattern. Many mechanical units are not appropriate to long periods of non-use. In addition to performance deterioration, increased maintenance often results from lack of use. So I think pretreatment simplicity is beneficial. Most importantly, the soil benefits from that type of use pattern.

Q: Gregg

This may be getting a little bit ahead of the schedule, but can you give me some idea of what percent of the outbreaks of disease that have been attributed to septic tank malfunctioning have been investigated, so that you feel reasonably sure what part of the system may be at fault.

A: Kreissl

In my paper I did present some data on this topic. The difficulty is that there are many outbreaks, as you know much better than I, where the source was not identifiable. The waterborne disease outbreaks where the source was identified in the period of 1970 through 1974 showed that less than 10 percent of the total were attributed to septic tank-soil absorption systems. Now, it is possible that these systems actually accounted for more than 10 percent of the outbreaks had the data been more complete. I noticed in the 1978 report from CDC that none of the 1978 outbreaks were identified as being from septic tank-soil absorption system (ST-SAS) sources. According to the CDC data for 1970 to 1974, the ten outbreaks caused by ST-SAS sources resulted from, in all but one case, etiological agents penetrating the unsaturated soil to the ground water with subsequent travel with the ground water to a drinking water supply. In the other case the system failed, surfaced, and overflowed down a hill to a well which was poorly sealed from surface pollution.

Q: Lennett

In discussing Figure 3 of your paper you mentioned something about one log reduction. Is this an across the board figure or a specific one?

A: Kreissl

That is just an average figure. In the case of Ascaris ova, you would expect to get two to three logs or more. In the case of a virus you may get negligible removal. My general statement is that you could not count on complete removal of any organism. The tank itself incorporates too many negative factors. As Dr. Rohlich points out, there is potential for significant short-circuiting. You also have digestion of solids which occurs in warm weather. These factors would negate consistently good removals of any organism.

Q: Lennette

Well, a reduction of one log is a 90 percent reduction, which to the engineers and chemists I guess is meaningful. To the microbiologist, however, that is not too meaningful. It is the absolute numbers which count.

A: Kreissl

I discussed this situation with Dr. Akin during the period I spent preparing the paper. I wish we had the data to properly calculate allowable pathogen levels at specific locations in the treatment systems for individual homes to permit control measures; something on the order of what Foster and Engelbrecht attempted, but with more accuracy.

Q: Pipes

I wonder if you could be more specific about the need for more information on pathogen removal. Do you have any specific pathogens in mind?

A: Kreissl

No, I don't. While rooting through the literature for the last few months, I found that there is very little definitive information which is useful to locations other than those where data were generated. For example, Cram's work on Ascaris in the 30's seemed to be most applicable, as did the Salmonella studies of Green and Beard of the same era. The lack of utility of many more recent studies to me may have related to my lack of ability to work in the microbiological literature, but I could not use some more recent studies because of a lack of necessary data on other controlling factors. The types of organisms in most need of study will be determined by groups such as yourselves. CDC documents the causative agents of waterborne disease outbreaks. Those are probably the ones with which to start. My feeling is that we just don't have useful information which can be applied to new sites for engineering analysis. The needed data must not only enumerate organisms, but it must also document hydraulic conditions, soil types and other physical and chemical phenomena. At present there are dots all over the place on organism removal at different locations. What we don't have is the scientific basis for putting them into a matrix that means something.

CURRENT PRACTICES IN LAND APPLICATION OF MUNICIPAL WASTEWATER

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ABSTRACT

Land application, also called soil treatment, is the practice of applying wastewater to the natural soil surface by one of three recognized processes--slow rate (irrigation), rapid infiltration, or overland flow. The primary objective is to utilize the natural soil properties and associated biological conditions for the removal or transformation of undesirable wastewater pollutants.

Each of the three categories of land application is currently at different stages of development. Because of their long history of use, slow rate systems are the most advanced. Good information is currently available for design and operation of these systems. The next most widely used is the rapid infiltration system. Because there is neither the number of systems in existence nor the background of experience available, design and operating criteria are not as well defined as for slow rate systems. The least developed of the three land treatment processes is the overland flow type. It is felt that this approach has great potential for municipal wastewater treatment, especially for small cities.

Slow rate systems are adaptable to a wide range of slopes and soil types. They are generally capable of producing a high quality treated water when well-designed and well-operated. In rapid infiltration most of the applied wastewater percolates through the soil, and the treated effluent eventually reaches the groundwater. The wastewater is applied to rapidly permeable soils, such as sands and loamy sands, by spreading in basins or by sprinkling. Removals of wastewater constituents by the filtering and straining action of the soil are excellent. Nitrogen removals are generally poor unless specific operating procedures are established to maximize denitrification. At overland flow systems, wastewater is applied over the upper reaches of sloped terraces by sprinkler or surface distribution systems and allowed to flow across the vegetated surface to runoff collection ditches. These systems are adapted to soils of slow permeability or sites which have a subsurface barrier; therefore, there is very little percolation and most of the water leaves the site as surface runoff. BOD and suspended solids should easily average 10 mg/l, or less, in the treated water. Total nitrogen in treated water can be expected to be less than 5 mg/l in a well-designed and operated system. Phosphorus removals usually range from 30 to 60% unless special treatment is employed.

The use of land application processes for treatment of municipal wastewater is growing in popularity and is encouraged by recent federal legislation and EPA policy.

CURRENT PRACTICES IN LAND APPLICATION OF MUNICIPAL WASTEWATER

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INTRODUCTION

Land application, also called soil treatment, is the practice of applying wastewater to the natural soil surface by one of three recognized processes--slow rate (irrigation), rapid infiltration, or overland flow. Each of these systems has well-defined differences regarding land area requirements, mode of operation, and resulting interactions with the plant, soil, and water components of localized ecosystems. Land application of municipal wastewater is normally preceded by some form of pretreatment. This may be either primary or secondary treatment. In some situations the application of raw wastewater may be considered. The primary objective is to utilize the natural soil properties and associated biological conditions for the removal or transformation of undesirable wastewater pollutants. A complementary interest is the beneficial utilization of the recycled water and other valuable components such as plant nutrients. This reuse feature of land application may result in such benefits as production of crops and livestock, recharge of groundwaters, and conservation of water and other wastewater constituents. The foremost consideration in land application is the treatment of the wastewater to meet water quality standards and other pollution control requirements.

Within the past decade, there has been a renewed interest in land application of wastewaters. This has resulted from several factors. Whether coincidental or not, the start of this new interest appears to be closely related to the widespread concern for environmental issues which arose in the mid to late 1960's. As a result of the renewed interest in land application in the United States, several things have occurred which have given added impetus and encouragement to its use.

The Federal Water Pollution Control Act Amendments of 1972 declared: "it is the national goal that the discharge of pollutants into the navigable waters be eliminated by 1985." The act also provided that the Administrator of the Environmental Protection Agency (EPA) "shall encourage waste treatment management which results in the construction of revenue producing facilities providing for the recycling of potential sewage pollutants through the production of agriculture, silviculture, or aquaculture products, or any combination thereof." Following the passage of this legislation, the EPA adopted the policy that land application must be evaluated as an alternative for all wastewater treatment systems funded under its Construction Grants Program.

In October of 1977, the EPA Administrator issued a much stronger policy statement which stated that ". . . the Agency will press vigorously for publicly owned treatment works to utilize land treatment processes to reclaim and recycle municipal wastewaters."

The most recent impetus to utilizing land application came with the passage of the 1977 Amendments to the Federal Water Pollution Control Act. These amendments include the provision that the EPA ". . . Administrator shall not make grants. . . to any state, municipality, or intermunicipal or interstate agency for. . . treatment works unless the grant applicant has satisfactorily demonstrated. . . that innovative and alternative wastewater treatment processes and techniques which provide for the reclaiming and reuse of water, otherwise eliminate the discharge of pollutants, and utilize recycling techniques, land treatment, new or improved methods of waste treatment management for municipal and industrial waste . . . have been fully studied and evaluated. . . ." The act further provides for certain monetary incentive for the construction of land application systems. So our country is strongly committed to the use of land application where feasible and appropriate.

PROCESSES

Each of the three categories of land application is currently at different stages of development. Because of their long history of use, slow rate systems are the most advanced. Good information is currently available for design and operation of these systems.

The next most widely used is the rapid infiltration system. Several systems of this type are in existence which have long histories of operation. Because there is neither the number of systems in existence nor the background of experience available, design and operating criteria are not as well defined as for slow rate systems.

The least developed of the three land treatment processes is the overland flow type. This type has been used to some extent with industrial wastes, particularly for food processing wastes, but has had very limited use for municipal wastes in the United States. It is felt that this approach has great potential for municipal wastewater treatment, especially for small cities. There is, indeed, a growing interest in overland flow and several systems are currently being planned and funded within the construction grants program.

Slow Rate

Design and Operation

Slow rate systems can be operated to achieve a number of objectives including: (1) treatment of applied wastewater, (2) economic return from use of water and nutrients to produce marketable crops (irrigation), (3) water conservation, by replacing potable water with treated effluent, for irrigating landscaped areas, such as golf courses, and (4) preservation and enlargement of greenbelts and open space. The applied wastewater is treated as it flows through the soil matrix. A portion of the flow percolates to the groundwater, and surface runoff of the applied water is generally not allowed. Figure 1 shows the features of a typical system.

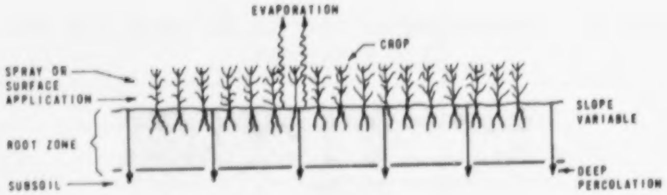

Figure 1. Slow Rate (Irrigation). Taken from Wastewater Treatment and Reuse by Land Application - Volume I - Summary, EPA-660/2-73-006a.

Slow rate systems are adaptable to a wide range of slopes. Slopes should be less than 20% on cultivated land but can be as great as 40% on noncultivated land. Soils range from moderately slow permeability to moderately rapid, and the minimum depth to ground water should be 2 to 3 feet. Storage capacity will be required for periods when water cannot be applied, such as during cold weather and rainfall events. Site characteristics are indicated in Table I.

Table II lists selected design features. Annual application may range from 2 to 20 feet per year being applied at a weekly rate of 0.5 to 4 inches. Methods of application are those normally used in agricultural practice including sprinkler systems and various types of surface application techniques. Minimum preapplication treatment in the United States is usually primary sedimentation but higher degrees of treatment may be required depending on types of crop and other requirements. Most of the applied water is lost to evapotranspiration and percolation. A crop is naturally an integral feature of slow rate systems.

Treatment and Performance

Slow rate systems are generally capable of producing a high quality treated water when well-designed and well-operated. Organics are reduced substantially in slow rate land application by biological oxidation within the top few inches of soil. Filtration and adsorption are the initial mechanisms in BOD removal, but biological oxidation is the ultimate treatment mechanism. BOD of the renovated water can generally be expected to average 2 mg/l or less. Suspended solids removals to 1 mg/l or less can generally be expected. Filtration is the major removal mechanism for suspended solids.

Nitrogen is removed primarily by crop uptake, which varies with the type of crop grown and the crop yield. To remove the nitrogen effectively, the portion of the crop that contains the nitrogen must be physically removed from the field. Denitrification can also be significant, even if the soil is in an aerobic condition most of the time. Average total nitrogen in the treated water can be expected to be about 3 mg/l with ammonia nitrogen less than 0.5 mg/l.

Phosphorus is removed from solution by fixation processes in the soil, such as adsorption and chemical precipitation. Removal efficiencies are generally very high for slow rate systems and are usually more dependent on the soil properties than on the concentration of the phosphorus

TABLE I. COMPARISON OF SITE CHARACTERISTICS FOR LAND TREATMENT

	Principal processes		
Characteristics	Slow rate	Rapid infiltration	Overland flow
Slope	Less than 20% on cultivated land; less than 40% on noncultivated land	Not critical; excessive slopes require much earthwork	Finish slopes 2 to 8%
Soil permeability	Moderately slow to moderately rapid	Rapid (sands, loamy sands)	Slow (clays, silts, and soils with impermeable barriers)
Depth to groundwater	2 to 3 ft (minimum)	10 ft (lesser depths are acceptable where underdrainage is provided)	Not critical
Climatic restrictions	Storage often needed for cold weather and precipitation	None (possibly modify operation in cold weather)	Storage often needed for cold weather

1 ft = 0.305 m

Adapted from Process Design Manual for Land Treatment of Municipal Waste-Water, EPA-625/1-77-008.

applied. A small but significant portion of the phosphorus applied (15 to 30% depending on the soil and the crop) is taken up and removed with the crop. Total phosphorus can be expected to average less than 0.1 mg/l in treated water. Table III presents a summary of expected treatment performance results for slow rate systems.

Rapid Infiltration

Design and Operation

The principal objective of rapid infiltration is wastewater treatment. Objectives for the treated water can include: (1) groundwater recharge; (2) recovery of renovated water by wells or underdrains with subsequent

TABLE II. COMPARISON OF DESIGN FEATURES FOR LAND TREATMENT

	Principal processes		
Feature	Slow rate	Rapid infiltration	Overland Flow
Application techniques	Sprinkler or surface[a]	Usually surface	Sprinkler or surface
Annual application rate, ft	2 to 20	20 to 560	10 to 70
Field area required, acres/MGD[b]	56 to 560	2 to 56	16 to 110
Typical weekly application rate, in	0.5 to 4	4 to 120	2.5 to 6[c] 6 to 16[d]
Minimum preapplication treatment provided in the United States	Primary sedimentation[e]	Primary sedimentation	Screening and grit removal
Disposition of applied wastewater	Evapotranspiration and percolation	Mainly percolation	Surface runoff and evapotranspiration with some percolation
Need for vegetation	Required	Optional	Required

a. Includes ridge-and-furrow and border strip.

b. Field area does not include buffer area, roads, or ditches.

c. Range for application of screened wastewater

d. Range for application of lagoon and secondary effluent

e. Depends on the use of the effluent and the type of crop

1 in = 2.54 cm
1 ft = 0.305 m
1 acre = 0.405 ha

Adapted from Process Design Manual for Land Treatment of Municipal Waste-Water, EPA-625/1-77-008.

TABLE III. EXPECTED QUALITY OF TREATED WATER FROM LAND TREATMENT PROCESSES (mg/1)

Constituent	Slow rate[a] Average	Slow rate[a] Maximum	Rapid infiltration[b] Average	Rapid infiltration[b] Maximum	Overland flow[c] Average	Overland flow[c] Maximum
BOD	<2	<5	2	<5	10	<15
Suspended Solids	<1	<5	2	<5	10	<20
Ammonia nitrogen as N	<0.5	<2	0.5	<2	0.8	<2
Total nitrogen as N	3	<8	10	<20	3	<5
Total phosphorus as P	<0.1	<0.3	1	<5	4	<6

a. Percolation of primary or secondary effluent through 5 ft (1.5 m) of soil.

b. Percolation of primary or secondary effluent through 15 ft (4.5 m) of soil.

c. Runoff of comminuted municipal wastewater over about 150 ft (45 m) of slope.

Adapted from Process Design Manual for Land Treatment of Municipal Wastewater, EPA-625/1-77-008.

reuse or discharge; (3) recharge of surface streams by interception of groundwater; and (4) temporary storage of renovated water in the aquifer.

In rapid infiltration systems, most of the applied wastewater percolates through the soil, and the treated effluent eventually reaches the groundwater. The wastewater is applied to rapidly permeable soils, such as sands and loamy sands, by spreading in basins or by sprinkling, and is treated as it travels through the soil matrix. Vegetation is not usually used, but there are some exceptions. Typical features are indicated in Figure 2.

The slope of the land is not critical; however, excessive slopes can increase costs because of increased earthwork requirements. Rapid infiltration systems can usually be operated year-round in most climates, greatly reducing or even eliminating storage requirements. If underdrains are not provided a minimum of 10 feet of soil column is required above

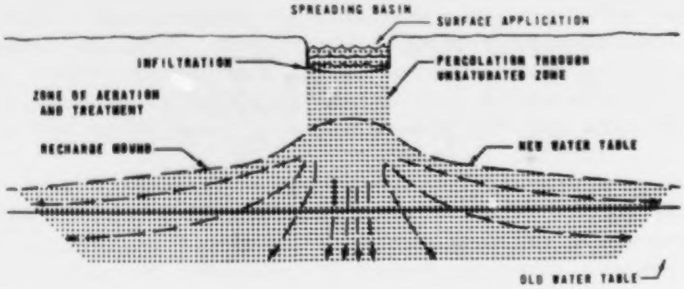

Figure 2. Rapid Infiltration. Taken from Wastewater Treatment and Reuse by Land Application - Volume I - Summary, EPA-660/2-73-006a.

the groundwater. Annual application rates can be in excess of 500 feet per year with typical weekly rates ranging between 4 and 120 inches. Primary sedimentation is the minimum preapplication treatment required to prevent soil clogging and other operating problems. In many cases, recovery of renovated water is an integral part of the system. This can be accomplished using underdrains or wells. Selected site characteristics and design features are presented in Table I and II, respectively.

Treatment Performance

Removals of wastewater constituents by the filtering and straining action of the soil are excellent. Suspended solids, BOD, and fecal coliforms are almost completely removed in most cases.

Nitrogen removals are generally poor unless specific operating procedures are established to maximize denitrification. Management practices to maximize nitrogen removal include: (1) adjusting application cycles, (2) supplying an additional carbon source, (3) using vegetated basins, (4) recycling the portions of the renovated water containing high nitrate concentrations, and (5) reducing application rates. With good management, total nitrogen in the treated water can average about 10 mg/l.

Phosphorus removals can range from 70 to 99%, depending on the physical and chemical characteristics of the soil. As with slow rate systems, the primary removal mechanism is adsorption with some chemical precipitation, so the long-term capacity is limited by the mass of soil in contact with the wastewater. Phosphorus concentrations in treated water can be expected to be less than 5 mg/l and average about 1 mg/l. Expected treatment results are presented in Table III.

Overland Flow

Design and Operation

The objectives of overland flow are wastewater treatment and, to a minor extent, crop production. Treatment objectives may be either (1) to achieve secondary or better effluent quality from screened primary treated, or lagoon treated wastewater, or (2) to achieve high levels of nitrogen and BOD removals comparable to conventional advanced wastewater treatment from secondary treated wastewater. Treated water is collected at the toe of the overland flow slopes and can be either reused or discharged to surface water. Overland flow can also be used for production of forage grasses and the preservation of greenbelts and open space.

At overland flow systems, wastewater is applied over the upper reaches of sloped terraces by sprinkler or surface distribution systems and allowed to flow across the vegetated surface to runoff collection ditches. As shown in Figure 3, the wastewater is renovated by physical, chemical, and biological means as it flows in a thin film down the relatively impermeable slope. Slopes may range from 2 to 8%.

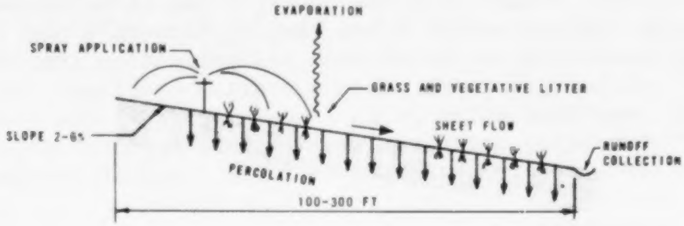

Figure 3. Overland Flow. Taken from Wastewater Treatment and Reuse by Land Application - Volume I - Summary, EPA-660/2-73-006a.

These systems are adapted to soils of slow permeability or sites which have a subsurface barrier; therefore, there is very little percolation and most of the water leaves the site as surface runoff with some evapotranspiration occurring. Because percolation is restricted, depth to groundwater is not a critical factor. Storage may be required in cold climates.

The annual application rate usually ranges between 10 and 70 feet. Weekly application rates have been reported over the wide range of 2.5 to 16 inches. The application rate is influenced to a great extent by the degree of preapplication treatment. The lower rates were reported from operations applying wastewater which had received only comminution and screening. The highest rates were from systems receiving secondary treated effluents.

Table I and Table II present pertinent site characteristics and design features.

Treatment Performance

Biological oxidation, sedimentation, and grass filtration are the primary removal mechanisms for organics and suspended solids. BOD and suspended solids should easily average 10 mg/1, or less, in the treated water, depending on degree of preapplication treatment.

Nitrogen removal is attributed primarily to denitrification. Because this process depends on two stages of microbial activity, it is sensitive to environmental conditions. Plant uptake of nitrogen can also be a significant removal mechanism. Permanent nitrogen removal by plant uptake is only possible if the crop is harvested and removed from the field. Ammonia volatilization can be significant if the pH of the water is above 7. Nitrogen removals usually range from 75 to 90% with runoff nitrogen being mostly in the nitrate form. Higher levels of nitrate and ammonium may occur during cold weather as a result of reduced biological activity and limited plant uptake. Total nitrogen in treated water can be expected to be less than 5 mg/l with an average of about 3 mg/l in a well-designed and operated system.

Phosphorus is removed by adsorption and precipitation in essentially the same manner as with the slow rate and rapid infiltration methods. Treatment efficiencies are somewhat limited because of the incomplete contact between the wastewater and the adsorption sites within the soil. Phosphorus removals usually range from 30 to 60% on a concentration basis. Increased removals may be obtained by adding alum or ferric chloride prior to application. With alum addition 90% phosphorus removal has been reported in the treated water.

Refer to Table III for a summary of expected treatment results from overland flow for certain wastewater constituents.

SUMMARY

The use of land application processes for treatment of municipal wastewater is growing in popularity and is encouraged by recent federal legislation and EPA policy. The three types of systems are at different stages of development with the slow rate process being the most advanced, followed by rapid infiltration, and overland flow in that order.

While the design and operating criteria for rapid infiltration and overland flow are not as well defined as for the slow rate process, all three systems can be designed for successful operation with certain limitations regarding management practices and climatic conditions.

The primary objective of any land application system is the treatment of the wastewater to achieve water quality requirements. Secondary benefits can include production of a marketable crop; conservation of water and valuable wastewater constituents; recharge of groundwater; and creation of parks, recreational areas, and greenbelts. When properly designed and operated, land application systems are a viable alternative to mechanical processes, from a performance standpoint, and are often more cost effective, less energy intensive, and simpler in operation.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

CURRENT PRACTICES - LAND APPLICATION: CURTIS HARLIN

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: Gregg

It is very difficult for those of us who know nothing about this area to visualize what a proposed or actually functioning land application system would look like. The acreage, the location, could you give me some sort of quick, visible idea of what we are talking about?

A: Harlin

In the paper based on the wide-range of loading rates presented there, you will find the area in acres required per 1 million gallons per day flow. These are very wide because the application rates are very wide. Just to give you some idea, some perspective, the largest land area requirements are for the slow rate system, the irrigation type system, and based on annual application rates of 2 to 20 feet per year land requirements would be 56 to 560 acres per million gallons per day flow. Now on the other hand, the rapid infiltration system, which requires the smallest acreage, we are talking about 2 to 56 acres per one million gallons a day flow. It is conceivable that the number of acres required for a rapid infiltration system, with very high loading rates, may not be much in excess of what you would need for a mechanical treatment site.

Q: Gregg

What is one million gallons per day related to in terms of human population?

A: Harlin

Approximately 10,000 population.

Q: Morrison

Dr. Harlin, on the rapid infiltration method can you speculate what kind of a protective acreage would be needed for other populations who would be driving wells. Obviously that kind of a system is not protecting the ground water in the immediate vicinity, from organic and biological pollutants.

A: Harlin

This answer is used so often that I hesitate to use it because it seems like a cop-out, but these systems are extremelly site specific and what you are referring to is the distance of travel from the site in the ground water to adjacent areas where you might contaminate wells. Of course, this depends on the gradient, the movement of the water in the subsurface, the degree of treatment that the water will receive as it percolates through the soil. I can't give you any specific figures, but some states in their state requirements do have numbers for them and they cover a very wide range so I think the only way I can answer this would depend on the very detailed hydrological survey of the area to determine the direction of flow and rate of flow and then an evaluation of the degree of treatment that water received before it reached that well.

Q: Morrison

Your answer is fine, but I wanted to get this into context of the individual home system where at least one family or small group of families are concerned with the way their wells are going to function. We now are dealing with a municipal center or industrial center. Are the consumer wells around it in somewhat of a distant risk or hazard situation? I would like to make one other quick comment without exploring it any further; that is you commented that these systems can be conservation measures and I do not completely agree that disposing of water in this manner is strictly a conservation measure. We are not making any new water and what might be perceived as conservation by one person is the holding back of water from somebody downstream or altering flood plain or wet land waters so I think we have to be careful on the generalized use of the word conservation.

A: Harlin

In the legislation both of the '72 amendments and the '77 amendments, they do refer to conservation and reuse. What is conservation to one person using the water for irrigation to replace other water sources might not be conservation to someone down stream who had been receiving that water prior to this land application. There is a big difference between the legislative view and a scientific view.

Q: Rycheck

Dr. Harlin, you mentioned management problems. First of all, what are the deleterious effects that you are alluding to as the result of management problems, and second, specifically what are the problems?

A: Harlin

Let's talk about the three different systems. The management problems that I referred to are different for the three systems. First of all, one of the major management problems in the slow rate system, that is irrigation, has to do with the possible conflict between the agricultural operations and the treatment aspects of the system. Sometimes these are not compatible and the agriculturalist would want to make the maximum use of the water to maximize his crop production. However, the person that is responsible for treatment of the water wants to maximize the treatment, so somewhere in between these needs, there can be, we think, management of the systems to maximize the treatment and still optimize the agricultural aspects. Now that's the primary thing in the slow rate system. Work needs to be done on optimizing the agricultural aspect without detriment to the treatment aspects. Work is being done with multi-cropping systems and this sort of thing. Now then, as far as the rapid infiltration system, the primary management problem we see is maximizing the nitrogen removal. This is the most serious. High nitrate levels in the ground water, of course are undesirable, but there are ways and systems have been tried that we feel reduce the nitrates going to a ground water system to acceptable levels. I don't know of any particular management problems in the overland flow system, based on our limited operating experience. Phosphorus removal is one, and significant work has been done on that.

Q: Rohlich

Could I ask one question, Curtis. You covered the quality of the treated water from these three different processes. Have there been any control studies made using these three different processes on the microbiological characteristics of the treated water and that which reaches the ground water?

A: Harlin

Jerry, there has been very little done in that compared to the other conventional parameters. In cooperation with the ground water program in Ada we have done some studies on viruses in ground water under land treatment systems. There have been scattered studies on bacteria and viruses penetrating the soil under these. Chuck Sorber has been involved in some of these. Gerba has been involved in some others. I guess you'll hear about this later. But data are not nearly as complete in this area, Jerry, as in your conventional treatment. I guess at this point, other than the effectiveness of these systems on organics and toxic substances, this is one of the greatest deficiencies.

BACKGROUND INFORMATION ON SOIL AND GEOHYDROLOGICAL CONSIDERATIONS

MICROBIAL HEALTH CONSIDERATIONS OF SOIL DISPOSAL OF DOMESTIC WASTEWATERS: SOIL CONSIDERATIONS

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ABSTRACT

Land application can be an economical and effective way to utilize wastewater if land treatment systems and management practices are carefully designed to avoid contamination of water resources and food supplies. Relatively thin layers of soils remove bacteria primarily by straining and viruses by adsorption. Exceptions are very coarse sands and gravels that have very high infiltration rates and may allow direct channeling to the groundwater. The most important soil characteristic affecting pathogen removal by soils seems to be soil permeability. The pathogen removal capacity of a soil can be changed by changing soil permeability and perhaps by other alterations. More research is needed to understand basic pathogen-soil interactions and to develop methods to increase the pathogen removal capacity of soils.

Land application of wastewater has received much attention because it offers a unique opportunity to turn wastewater into a valuable water resource while minimizing the pollution of surface water. Since wastewaters contain a wide variety of pathogenic bacteria, viruses, protozoa, and parasitic worms, care must be taken to design land treatment systems so that the risk of human exposure to these pathogens is minimized.

Some pathogens can be removed by conventional sewage treatment prior to land disposal. Foster and Engelbrecht (14) assessed the pathogen removal efficiency of conventional sewage treatment systems (Table 1). In general, primary treatment reduces the populations of helminth ova but has little impact on numbers of bacteria or amoebic cysts. Virus removal is probably significant because most viruses are associated with suspended particles, although some laboratory studies have failed to demonstrate significant virus removal. Thus, primary treatment removes only those pathogens heavy enough to settle out of the water or associated with suspended solids. However, primary treatment may have a significant impact on pathogen survival on crops and soils due to removal of suspended solids which protect pathogens from adverse conditions.

Activated sludge treatment results in about 99% removal of bacteria and viruses but little additional removal of amoebic cysts or helminth ova. Removal by trickling filters can be more efficient with up to 99.9% removal of bacterial pathogens and amoebic cysts and up to about 75% removal of helminth ova and viruses. Thus, some pathogens can be removed by pretreatment, but management practices and exclusion of certain uses are usually more practical and cost effective than pretreatment (26). The effect of soil characteristics on pathogen

TABLE 1. REMOVAL OF PATHOGENS FROM SEWAGE WATER BY CONVENTIONAL TREATMENT SYSTEMS*

Pathogen	Primary	Activated Sludge	Trickling Filter
	%		%
Salmonella	15	96 to 99	84 to 99.9
Mycobacterium	48 to 57	Slight to 87	66 to 99
Amoebic cysts	No removal in 3 hrs.	No apparent removal	11 to 99.9
Helminth ova	72 to 98	No apparent removal	62 to 76
Virus	3 to extensive removal	76 to 99	0 to 84

*Adapted from Foster and Engelbrecht (14).

removal during land disposal is one of the more important factors to consider in the design of management practices.

SELECTION OF LAND TREATMENT SYSTEMS

One of the more important ways that soil characteristics affect land treatment systems is in the initial design. The kind of land treatment system selected is determined largely by the kind of soil available. Only overland flow systems are used where soils with low permeability are available. These systems are usually on fine textured soils designated as clays.

Irrigation systems usually are used on medium textured soils with moderate infiltration rates and high water holding capacities. Sandy loam or loam soils usually provide these characteristics. Typical application rates range from 2 to 10 cm per week. High rate or groundwater recharge systems are used on highly permeable soils such as sands and loamy sand. Application rates for high rate systems vary from about 0.5 to 8 m per week. More details on the selection of soils for land treatment systems are available in the Process Design Manual for Land Treatment of Wastewater (35). The different land treatment systems present different potential hazards from contamination by pathogens (Table 2). The main ones influenced by soil characteristics are food contamination (due to the effect of soil characteristics on pathogen survival in soils) and groundwater pollution.

EFFECT OF SOIL CHARACTERISTICS ON PATHOGEN SURVIVAL

After removal from wastewater, bacteria may survive in the soil from a few hours to several months (6, 15). Since the enteric bacteria must compete with other micro-organisms in the soil, survival is regulated by a complex ecological balance that can be affected in many different ways by changing environmental conditions. In general, survival is enhanced by high soil moisture contents, low soil temperatures, high pH, the presence of soil organic matter, and shielding from sunlight. Usually, bacterial pathogens are reduced to negligible numbers after 2 to 3 months (15), but bacteria can survive many months at freezing temperatures. Thus, fine textured soils with high moisture-holding capacities favor bacterial survival due to the direct effect of the higher water content and to the retardation of soil warming in the spring by high water contents.

Virus survival in soils is also directly related to temperature. In laboratory studies, Leffler and Kott (30) and Dubiose et al. (12) found that poliovirus added to sand columns survived about 90 days stored at 18 to 20°C. However, 69 to 90% of the virus was inactivated after 7 days. Survival time was greatly prolonged at 4°C, with viruses still surviving after 175 days.

Yeager and O'Brien (42) using radioactivity labeled poliovirus and coxsackie virus B1, recorded no infectivity from saturated soil after 12 days at 37°C; whereas viruses persisted for 180 days at 4°C. Similarly in field studies, Tierney (34) reported that seeded poliovirus survived for 11 days during the summer and 96 days during the winter in soils irrigated with sewage.

TABLE 2. POTENTIAL CONTAMINATION BY PATHOGENS ASSOCIATED WITH DIFFERENT LAND TREATMENT SYSTEMS

Types of Land Treatment Systems	Problems Associated with Land Treatment			
	Food Contamination	Ground-water Pollution	Surface Water Pollution	Aerosols
Overland flow	-	-	++	+
Irrigation (slow rate	+	+	+	++
Rapid infiltration	-	++	-	-

- Little or no potential problem
+ Moderate potential
++ Considerable potential

Moisture content has little direct effect on virus survival except at very low moisture contents. Yeager and O'Brien (42) reported that infectivity of soil samples dried in a desiccator decreased only slightly as the moisture content decreased from 18 to 2% over a 4-hour period. However, infectivity was considerably reduced at moisture contents of 1.0 and 0.6%. This shows that soils must be dried to very low moisture contents to inactivate viruses directly. The direct drying effect would seldom occur under field conditions because soils in the natural state seldom dry to a 2% moisture content, particularly in land treatment systems where drying periods are usually limited to a few days. The soil surface can become very dry, but even at the surface, the viruses are probably inactivated before the low moisture content is reached. Hurst et al. (20) found that viruses could not be recovered after 5 days drying from the surface of a basin used to recharge primary sewage effluent even though the moisture content was 8%. However, moisture content has a very important indirect effect because virus survival is greatly increased by anaerobic conditions. In laboratory studies, Hurst et al (19) showed that viruses survived longer under anaerobic conditions than under aerobic conditions. Also, viruses survived much longer under sterile conditions than nonsterile conditions in an aerobic environment at 23°C. Survival times were similar for sterile and nonsterile soils under anaerobic conditions, which indicated that aerobic soil micro-organisms are antagonistic to viruses. Similarly, in field studies using both lab grown viruses seeded in the soil and soil samples from basins flooded with primary sewage effluent, Hurst et al. (20) showed that viruses survived longer during flooding cycles than during drying cycles and longer in closed tubes (buried in recharge basins) than in open tubes. The effect of drying cycles in the field probably was due to aeration of the soil rather than the soil drying per se because the soil moisture content usually exceeded 10% at the end of the drying period. Thus, the effect of soil drying is important because drying the soil allows growth of aerobic bacteria which evidently are antagonistic to viruses. Viruses could survive for months if they reach a section of the soil profile that is kept anaerobic by saturation. However, most viruses are adsorbed near the soil surface except in a few problem soils, as will be explained in detail in a later section. However, viruses can survive for long periods in wetlands used for land disposal and possibly in soils with very shallow groundwater tables.

EFFECT OF SOIL CHARACTERISTICS ON PATHOGEN MOVEMENT TO GROUNDWATER

As wastewater moves through the soil, bacteria are removed by straining, adsorption, and die-off. Clays in soils enhance adsorption. However, the smaller particle sizes in clay soils also result in more straining of bacteria at the surface. Thus, it is difficult to assess the relative importance of straining and adsorption. Butler et al. (8) found that removal of bacteria from liquid percolating through a given depth of soil was inversely proportional to the particle size of the soil and was highest during the first few centimeters of travel. He concluded that removal was by straining. Development of an organic mat on the surface enhanced the removal of coliforms. Obviously, slowly permeable soils would allow fewer bacteria to move through the soil than would move through coarse sands because the lower flow rate would allow more time for bacterial die-off. Bitton et al. (2) concluded that the sieving effect of the soil increases as the soil water content decreases. This indicates that bacterial movement through soils intermittently irrigated to grow crops should be much less than in recharge basins flooded for days or weeks.

Any situation that results in unsaturated flow through soils should result in less movement of bacteria than would result from saturated flow. Thus, fine textured soils transfer few bacteria not only because they strain bacteria at their highest infiltration rates but also because they are susceptible to clogging at the soil surface; thereby producing unsaturated flow and subsequently more bacterial straining. Soil clogging may be particularly important in increasing the effectiveness of bacterial straining by septic tank fields.

Unlike bacteria, most of the virus removal by soils is due to adsorption. A number of studies with various soils has shown that many soils have a high capacity to adsorb viruses (1, 15, 24, 29). Much of the work relating soil characteristics to virus adsorption has been done in experiments using small quantities of soil mixed with viruses suspended in solutions.

Some of these batch studies indicated that virus adsorption increased with increasing cation exchange capacity and specific surface area, and decreased with increasing pH (7, 11). However, in each study, adsorption to at least one kind of soil did not fit this pattern. For example, Drewry and Eliassen (11) found that a soil with a low cation exchange capacity was one of the higher in the test group for virus adsorption.

Other batch tests have indicated that virus adsorption by clays increased as the salt content of the suspending solution increased; adsorption could be reversed by suspending the clay in distilled water; adsorption varied for different kinds of clays; and proteinaceous materials like egg albumin interferred with virus adsorption (5, 9, 31).

However, in one extensive series of experiments using batch studies and 250-cm long soil columns, results from the batch studies did not always agree with the column results. In the batch studies, the adsorption of virus types and strains to nine different soils was compared using suspensions in deionized water (18). A great deal of variability among types and strains was noted, along with considerable variability among different soils. In general, some viruses, such as polio, adsorbed to all of the soils, whereas soils with a pH below the isoelectric point of the viruses had a high adsorption rate for all viruses. Soil pH was the only soil characteristic which consistently affected virus adsorption.

Then the experiments were continued in the soil columns using one soil, a loamy sand from the Salt River bed in Arizona, poliovirus that adsorbed well to that soil in batch studies, and two reference strains, Echo 1 and Echo 29, that adsorbed poorly to the soil in batch studies (27). The adsorption patterns of Echo 29 and Polio 1 were almost identical with 90% adsorption in the top 2 cm and no viruses detected below 160 cm. Adsorption of Echo 1 was 77% for the top 2 cm, but the adsorption pattern was similar to that of the other viruses below the 40-cm depth. Thus, more Echo 1 than Polio 1 viruses moved to the 40-cm depth, but numbers moving to lower depths were similar. The desorption and movement of the polio and echo viruses were similar when the column was flooded with deionized water. Thus, these experiments showed that although batch studies showed great differences in adsorption between strains, the columns that are realistic models of a field groundwater recharge system showed only slight differences in adsorption of the different virus strains. Therefore, one must be careful in extrapolating batch studies to field conditions. Thus far, the results from batch studies that have been confirmed in column and field studies are: salts enhance virus adsorption, some organic compounds interfere with virus adsorption, and distilled water desorbs viruses. Other work on the effect of soil characteristics and on differences among virus strains has not been confirmed. The organic content of secondary effluent causes less soil adsorption of viruses from secondary effluent than from tap water, but the higher organic content in primary effluent does not cause soil adsorption from primary effluent to be less than that from secondary effluent (16).

Both column and field studies have shown that soil permeability is one of the more important factors affecting virus adsorption. Some of the earlier work on virus migration through porous media centered on sand filters used in the treatment of drinking water. Robeck, et al. (32) allowed poliovirus suspended in dechlorinated tap water to percolate through 60 cm of California dune sand. With virus doses of from 10,000 to 60,000 PFU/ml (plaque-forming units/milliter), the sand was capable of removing 99% of the viruses for 98 days of operation. Virus removal was found to depend on flow rate through coarse sand; removal greater than 90% occurred when flow rates were below 1.3 m/day. Higher flow rates resulted in considerably more virus breakthrough.

Lance and Gerba (28) showed that viruses were not detected below 160 cm when a column packed with coarse sand was flooded with virus enriched sewage at a flow rate of 0.6 cm/day. However, some viruses broke through the column when the flow rate was increased to 1.2 m/day. Virus adsorption was not greatly affected by further increases in the flow rate. Apparently virus adsorption is not affected by increases in the flow rate up to some breakthrough point. After that breakthrough point was reached, some viruses moved through, but 99% was still removed at flow rates of 12 m/day. The breakthrough velocity probably corresponds to the velocity where some water begins to move only through the large soil pores, allowing little or no contact between viruses in the water and adsorptive surfaces. The breakpoint apparently occurred between 0.6 and 1.3 m/day for the Salt River bed sand columns, and at about 1.2 m/day for the sand columns used by Robeck et al (32), but it might be different for other soils. Vaughn et al. (37) found that virus movement through a test recharge basin was reduced as the basin infiltration rate was reduced due to surface clogging.

In adsorption studies with different soils packed into 87-cm columns, poliovirus and echovirus did not move below 67 cm in three columns but moved completely through another column (38, 39). Virus adsorption was inversely proportional to the permeability of the different soils; thus, these experiments on flow velocity suggested that permeability may be the most important soil characteristic affecting virus movement.

Field studies also have shown the effect and importance of soil permeability on virus movement. Only since the early 1970's have methods of concentrating viruses from large sample volumes been available for use in field studies of virus movement. Viruses have been detected in the groundwater below several field sites (Table 1) located on sandy or gravelly soils with high infiltration rates (23, 33, 36, 40, 41). One site at St. Petersburg, Florida, was located on a sand which probably had a high infiltration rate, and viruses were detected after a 3-month period when 70 cm of rainfall was recorded (40). Bitton et al. (3) showed that organic compounds interfered with virus adsorption when wastewater was released onto a Cypress dome in Florida.

In studies at field projects with finer textured soils and low infiltration rates, researchers have not detected viruses in samples from wells or lysimeter leachates (4, 10, 13, 17, 22). Concentrated duplicate samples ranging from 174 to 454 l from each of four wells at about 2-month intervals during a year of operation at the Flushing Meadows Project in Arizona did not yield any positive samples for viruses (17). The Flushing Meadows Project was a groundwater recharge system for renovation of secondary sewage effluent where infiltration rates ranged from 30 to 70 cm/day. The rainfall in the area is low, but soil column studies mentioned previously indicated that rainfall would have little effect on virus movement at the site (25). With a single questionable exception, viruses were not isolated from leachate samples collected from 150-cm-deep lysimeters located in grass sod and sugarcane fields in Hawaii that were regularly irrigated with secondary effluent (13). Studies at Roswell, New Mexico, also failed to yield viruses in 151- to 333-l samples taken from shallow wells (3 to 37 m) beneath farmland that was intermittently flooded with secondary sewage effluent (27). Concentrations of viruses in the Roswell wastewater before land application ranged from 960 to 5070 PFU/l.

Viruses added to sludge placed on lysimeters in Denmark did not move through the soil even though they survived for 23 weeks (10). Water movement was slow since no water was added to supplement the 30 cm of rainfall during the test period. Viruses were not detected in groundwater samples of 189 to 378 l from a site in Florida where sludge was spread on agricultural land (4). Probably viruses applied in sludge are less mobile than those applied in sewage water because of adsorption to sludge solids.

Thus, a summary of results from field studies indicates that virus movement to the groundwater is a problem primarily in soils composed of coarse sands and gravels that have very high permeabilities. Such soils may also allow channeling which permits passage of virus to groundwater without contact with the soil.

Thus, field and laboratory studies indicate that soil permeability may be the most important soil characteristic affecting virus adsorption, and virus movement can be reduced by reducing the permeability of a soil. One special case where another soil characteristic is very important may be soils where the pH is below the isoelectric point of viruses (usually below pH 5) resulting in very strong attraction between the negatively charged soil particles and positively charged viruses. Also, there is some evidence that iron oxides may have a strong affinity for viruses and, therefore, soils high in iron oxides may have a particularly high virus adsorption capacity.

Another special case may be swelling clay soils like the soils in the Blackland Prairie of Texas where large cracks or channels open in the soil during drying. Such soils allow leaching of salts and could allow penetration of pathogens to groundwater, particularly if they are above a shallow water table (21).

VIRUS-SOIL INTERACTIONS

Lance et al. found that most of the poliovirus suspended in secondary sewage effluent was adsorbed in the top 5 cm of the soil columns (25). The virus concentration in the sewage water was reduced by about 2 logs (from a starting concentration of 3 x 10^4 PFU/ml) during the first 2 cm of travel, but an additional 158 cm of travel was required for another 2 log reduction. When viruses were eluted from soil samples taken from groundwater recharge basins, the greatest amount of virus was found in the upper 2.5 cm of soil (20). The virus concentrations in the top 2.5-cm layer were about 10 to 15 times as high as those found in the 2.5- to 10-cm zone. This indicates that the virus adsorption pattern of the soil columns accurately represented the field adsorption pattern. Thus, the virus adsorption rate near the soil surface was greater than it was for deeper sections of the soil profile.

When a column packed with soil that had not previously been flooded with sewage water was flooded with virus enriched sewage, most of the viruses still were adsorbed near the soil surface (28). This showed that the concentration of viruses near the surface of the soil columns was not due to a buildup of organic material or salts near the soil surface. Also, since the columns were uniformly packed, the concentration of viruses near the surface was not due to different soil characteristics of the surface layer.

When a soil column was flooded with secondary effluent containing different concentrations of added poliovirus during different flooding periods, the number of viruses detected at every soil depth increased as the concentrations of added virus increased from about 0.9×10^2 to 2.6×10^4 PVU/ml. However, the percentage of added virus remaining at each depth was about the same for each concentration. In addition, increasing the concentration of added virus did not increase the maximum depth to which virus moved.

One possible explanation for the virus adsorption pattern in soil columns flooded with seeded sewage water is that virus adsorption is affected by the strength of the negative charge on the virus particle. Perhaps viruses with a net negative charge below a certain level are immediately adsorbed while viruses with a stronger negative charge move farther down the column. This would explain why the percent removal of virus at various depths is similar for applications of wastewater containing different virus concentrations. When a virus population is diluted to provide various concentrations, the percentage in each virus concentration having a particular charge strength would not change, and, therefore, the percent adsorption at each depth would not change either.

Virus adsorption to soil appears to be due to London-Van der Waals forces. Such adsorption is inhibited by the layer of negatively charged ions that surrounds both the soil particles and the viruses and causes them to repel each other. Thus, if the strength of the negative charge does vary among different viruses, their adsorption by soils would vary also.

Increasing the ionic strength of the soil solution decreases the thickness of the double layer (or cloud of ions) around the soil particles and viruses and promotes adsorption. Therefore, anything that changes the salt concentration of the soil solution affects the virus adsorption capacity of the soil. For example, virus adsorption increases as the salt content of the wastewater increases. Lance and Gerba (unpublished data) have found that some salts such as aluminum compounds are more effective than other salts in promoting virus adsorption.

SUMMARY

Site selection is one of the more important ways in which information on soil characteristics affect the development of land disposal systems to minimize microbial hazards. Soil characteristics (primarily permeability) determine the kind of site selected. Permeability is also one of the more important factors affecting pathogen movement through soils. Also, it is important to avoid using shallow soils for irrigation or rapid infiltration systems. In considering the effective depth of the surface layer where more of the pathogen removal occurs, one must consider the depth to a slowly permeable layer or the depth to a layer with much higher permeability and possible channels.

Some evidence indicates that the pathogen removal by soils (particularly viruses) can be improved by changing the infiltration rate. This can be done by using long flooding cycles to promote surface clogging, compacting the soil surface, and by reducing the depth of ponding in flooding basins. It is possible that the soil may be altered in other ways such as the addition of certain salts to the soil. However, there is not yet sufficient research to support other practices that alter the soil for improvement of its pathogen removal capacity.

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SOIL CONSIDERATION: J. C. LANCE

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: Pipes

What did you have in mind in terms of other practices?

A: Lance

There are various ways to apply wastewater to the soil. In some cases, water moves through the soil as saturated flow while in other cases, it moves as unsaturated flow. Since virus removal is more effective during unsaturated flow, changes in application procedures or in the soil profile that promote unsaturated flow would increase virus retention by the soil. The ions held on the cation exchange complex in the soil may affect virus retention. It is possible that adding certain salts to soils would increase virus retention. Finally, changes in the soil profile such as adding small amounts of clay to the soil or laying down a clay layer might be feasible practices to increase virus retention.

Q: Morrison

Have you considered the possibility of other anomalies that may be functioning with these viruses? The binding on surfaces? You didn't get a steady state increase. Have you considered the possibility that the surface of the soil has predators? I think one of the areas we overlook very often is the fact that soils do have larger biological protozoans, rotifers and so forth, possibly functioning as scavengers in the upper layers of the soil.

A: Lance

We did not consider the possibility that predators in the surface layer of soil might be responsible for the high removal rates near the surface. Since the flow rate through the soil was 60 cm/day, we did not think that would allow sufficient time for predators to affect virus movement. Water would move through the upper 5 cm of soil in a very short time.

We did experiment with a freshly packed uniform soil column that had never been treated with sewage before our experiments. We obtained the same adsorption pattern in that column. Therefore, since predators would not have been present in that column packed with dry soil, we don't believe that the virus removal or retention near the soil surface could have been due to the activity of predators.

Q: Morrison

I would like to follow up with just a comment on what Dr. Pipes alluded to. What about the differences in the ions in terms of the surface phenomena, the difference between trivalent, divalent, and monovalent ions of several metallics, distinctly affecting that surface? You showed that the tap water had a distinctive different adsorption than the distilled water. Could this be just due to the trace of ions?

A: Lance

The effect of tap water on virus adsorption is due to the ionic composition. We tried to plot virus-adsorption vs. virus-ionic strength because ionic strength is affected by both valence and concentration. Theoretically, this would allow a comparison of ions of different valences. For example, aluminum salts at a given concentration should have the same ionic strength as a potassium salt or a calculated higher concentration. This procedure gave some useful comparisons but also revealed some unexpected results. For example, solutions of aluminum salts were more effective than solutions of other salts even if the ionic strengths of the solutions were the same. For another example, ammonium and potassium are both monovalent ions, but the ammonium ion increased virus retention much more than the potassium ion at similar concentrations.

Q: Davidson

What do you consider a virus? Is it a point charge such as a cation (calcium or fluoride) or do you consider it a colloid? If you consider it a colloid then you should use double layer theory to describe the coagulation process. If you take this approach your answers are going to be much different than if you treat it as a point charge. If it is truly a flocculation or coagulation process, then you may not have a true equilibrium but a time dependent process.

A: Lance

We tried to apply double layer theory to virus adsorption and as I mentioned in the previous question, we were partially successful.

HYDROGEOLOGIC CONSIDERATIONS IN WASTE DISPOSAL

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ABSTRACT

Ground water has served as a source of supply for domestic and municipal needs for centuries, yet it seems to have been only recently discovered by many governmental agencies. The movement of underground water can be adequately described and predicted, but the fate and transport mechanisms of many pollutants is clouded by a lack of adequate data that relate to their attenuation. Some evidence indicates that the chemical quality of shallow aquifers can undergo rapid and substantial changes within days or even hours.

This paper discusses the hydrogeologic considerations of waste disposal. Included is a discussion of the mechanics of pollutant migration to the ground water, ground water flow, and transport of the pollutant within the ground water. The paper also discusses the various pollutant removal mechanisms of both the saturated and unsaturated zones and addresses the subject of well-casing interferences on ground water quality samples. A series of research recommendations is included at the end of the text.

INTRODUCTION

Water is the most essential substance on the earth, yet one of the most abused and at the same time one of the most destructive natural forces known to man. It's presence determines the value of lands and the wealth of nations. It has formed the basis of many religions and laws.

For centuries mankind has been concerned with water quality as well as quantity. Water pollution has been a vexing problem for centuries. Caeser's army carried boiled water during its middle Eastern campaigns because of dysentary brought about by local supplies. Even earlier, Hippocrates warned his Greek countrymen to filter and boil water before drinking it. He didn't know the underlying reason but he saw a correlation between certain illnesses and drinking water.

Ground water has recently been "discovered" by several governmental agencies, despite the fact that it has served as a major source of supply for centuries, particularly in arid and semiarid regions. Still rather esoteric, ground water to this day remains shrouded by a great deal of mystery and misunderstanding.

Underground water is an excellent resource due to its quality and availability. It is available at almost any lcoation in quantities large enough to provide for a single dwelling and more than a third of the Nation is underlain by aquifers capable of providing 100,000 gallons daily or more. The development of ground water supplies has little effect on land use and can be accomplished at relatively moderate costs, probably an order of magnitude less than surface water.

GROUND WATER STORAGE AND USE

Ground water in storage to a depth of a half mile within the 48 contiguous states has been estimated to be 180 billion acre-feet, which compares to 27 billion acre-feet contained in the larger lakes of North America. The total amount of ground water in storage, which is usable with today's technology, approximates 46 billion acre-feet, which equals 10 years annual precipitation or 35 years annual surface runoff.

Ground water, truly an ubiquitous resource is tapped by nearly 17 million wells and almost 500,000 new wells are constructed annually. Ground water supplies the domestic water needs of 20 of the Nation's largest one hundred cities and 96% of all rural America. One half of the total U.S. population relies on ground water for their domestic water supply. Of this amount, 60 million people are served by public water supplies and about 40 million use private wells.

GROUND WATER QUALITY

In any discussion of ground water quality it is essential to describe differences between natural quality and man-influenced quality. In the first case, natural ground water chemical quality is closely related to precipitation and, as a general rule, the least mineralized water occurs in areas of the greatest rainfall, which is generally along the coast. Inland precipitation decreases, water supplies diminish, and the quality deteriorates. Water bearing rocks exert a strong influence on ground water quality and, thus, the solubility of the rocks may override the role of precipitation.

Where precipitation exceeds 40 inches a year, shallow ground water usually contains less than 500 mg/l and commonly less than 250 mg/l of dissolved solids (Figure 1). Where rainfall ranges between 20 and 40 inches, dissolved solids may range between 400 and 1000 mg/l and in drier areas they commonly exceed 1000 mg/l.

Extremely hard water is found over most of the Interior Lowlands, Great Plains, Colorado Plateau, and Great Basin. Isolated areas of high hardness are found in northwestern New York, North Carolina, the southern tip of Florida, and parts of southern California. In general hardness is of the carbonate type.

On a regional level chloride does not appear to be a problem. Several small areas of high chloride concentrations appear along the coast of Florida, Virginia, North Carolina, Louisiana, Texas, and California. A broad belt of isolated chloride-rich areas extend through the center of the country from Alabama and New Mexico, through Oklahoma and Kansas, to South Dakota in the north.

In many locations sulfate levels exceed the federal recommended limit of 250 mg/l. Regional sulfate may be a problem only in the northern Great Plains, the eastern Colorado Plateau, Ohio and Indiana. Iron is ubiquitous and concentrations in excess of only 0.3 mg/l will cause staining of clothing and fixtures, while higher amounts produce an unpalatable, metallic taste. Excessive concentrations of iron in ground water is probably the single most widespread problem of domestic supply in rural America.

Fluoride concentrations are abnormally high in several areas, particularly in western Texas, western Oklahoma, and parts of several other western and midwestern states. Consumption of the high fluoride drinking water that are less than 1.4 mg/l in warm areas and less than 2.4 mg/l in cold areas is beneficial in the reduction of dental decay, especially among children.

The Nation is placing increasing reliance on ground water sources to provide its needs and it is particularly alarming that the quality of ground water is being degraded by a wide variety of man-made substances or through man's activities. This degradation is the result of a ground water use that is commonly not realized because it does not involve withdrawing water from a well. Nonetheless, to use ground water for waste disposal, whether inadvertently or deliberately, is as much a use as is withdrawal. Within the past 20 years a considerable

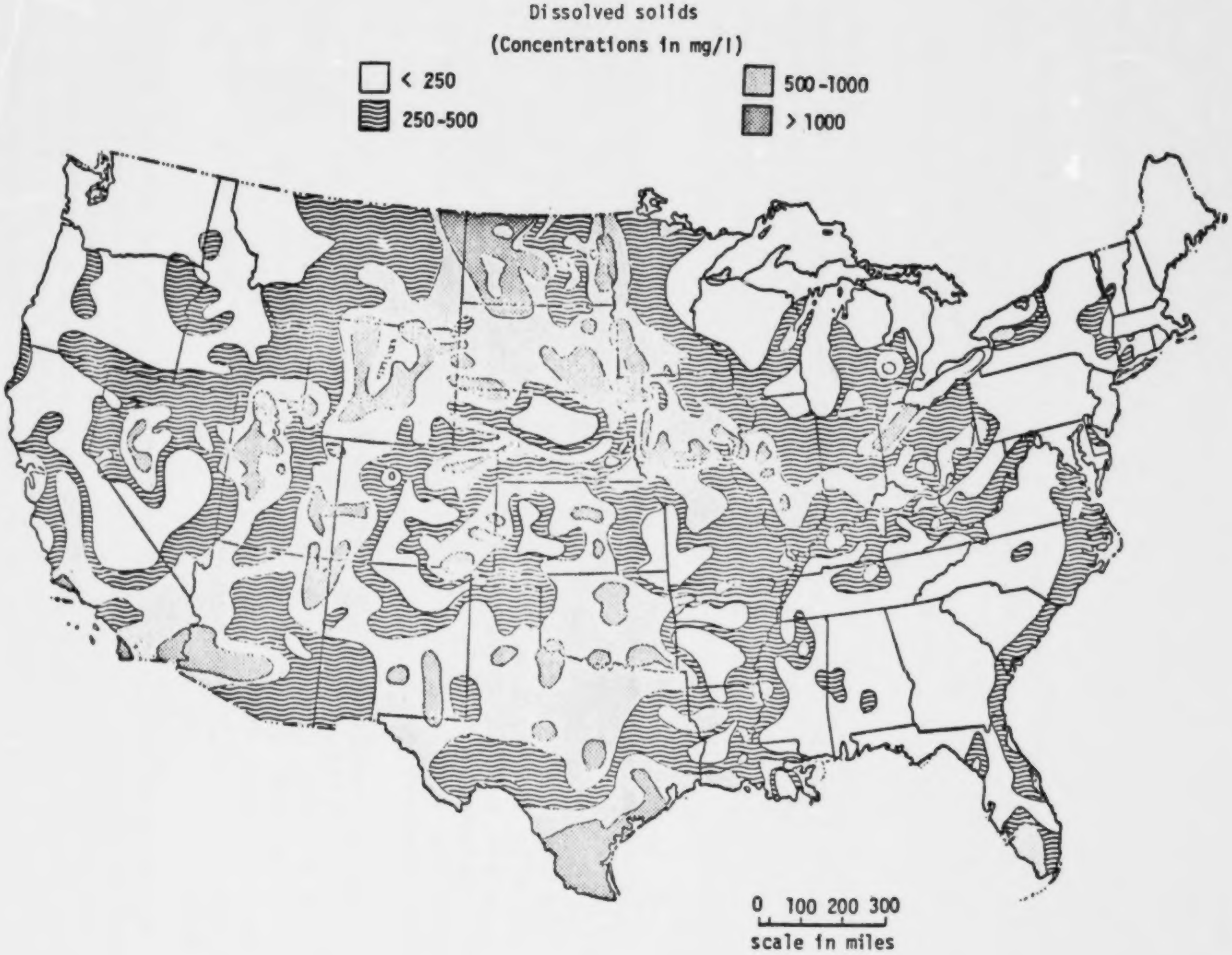
Dissolved solids
(Concentrations in mg/l)
< 250
250-500
500-1000
> 1000
0 100 200 300
scale in miles

Figure 1. Distribution of Dissolved Solids in Drinking Water Obtained from Ground Water Sources.

number of reports covering various geographical areas of the United States have included discussions of waste disposal practices and their effects on ground water quality.

Some activities, such as highway deicing, are unique to certain parts of the country, while others are common to all areas. Thirty percent of the population uses septic tanks or other means of subsurface disposal of domestic wastes. About 300 billion gallons of such waste are discharged each years. Similar waste discharges from commercial and industrial establishments further add to the volume.

Landfills and waste impoundments pose a threat to ground water quality that is only now being understood, given the complexities of measuring and identifying constituents of the leachate from these facilities. Recent investigations indicate that a substantial percentage of the industrial landfills contaminate ground water with organic chemicals.

It should be pointed out that ground water pollution generally affects only a small area, such as an individual well, a few acres or a segment of a stream. On the other hand, highly mineralized or unusual water of natural origin may underlie hundreds or even thousands of square miles and render these supplies useless for many purposes without extensive treatment. Therefore it is important to put into its proper prospective the national impact of ground water pollution relative to natural water quality.

FUNDAMENTALS OF GROUND WATER FLOW

From a hydrogeological viewpoint the subsurface is divided into two distinct zones: (1) the unsaturated zone, which extends from land surface to some depth where the water table is encountered and (2) the saturated zone, which extends downward from the water table. The water table, although it may fluctuate several feet each year, is the boundary between these two major zones. Openings in rocks are filled largely with gases in the unsaturated zone, while in the zone of saturation they are filled with water.

Commonly, although not every case, waste liquids must migrate through the unsaturated zone in order to contaminate ground water. The unsaturated zone is exceedingly important because it is here that many chemical and biological reactions occur that attenuate wastes. Unfortunately undue reliance has been placed on the "living filter" concept of the unsaturated zone, with the assumption that biological degradation and adsorption will attenuate wastes to such a degree that ground water contamination is not likely to occur. The fallacy of this argument is well substantiated by the thousands of ground water contamination cases that have been reported. Nonetheless, the unsaturated zone is a major control of ground water contamination because it exerts such a strong influence on the movement of biodegradable and nonconservative substances. Obviously the longer it takes a waste to migrate through the unsaturated zone, the more time and mechanisms there are to attenuate it.

In addition to thickness, the major controlling factors of the unsaturated zone are permeability, grain size, and chemical composition of the earth materials. Generally speaking those materials that have a high ion exchange capacity, a low permeability and consist of fine-grained materials will have the greatest attenuation affects. Therefore, the thickness and composition of the unsaturated zone must be examined in any waste disposal scheme.

In its simplest form, the movement of ground water can be described by means of Darcy's law, that is:

$$Q = KIA$$

Where Q = the flow rate, K = permeability, I = hydraulic gradient, and A = the cross sectional area through which the flow occurs.

Permeability refers to the interconnection of pore spaces and ease with which water can move from one point to another. It ranges within wide extremes, being greater for coarse materials than for fine. Permeability is divided into two types: primary and secondary. In the first case the permeability came into being when the strata were deposited and/or lithified, but secondary permeability originated after the rocks were lithified. Secondary permeability includes fractures and solution openings.

The hydraulic gradient is an expression of the slope of the water table and the difference in head or water level is a function of the amount of energy used as the water moves from one point to another. A water-level map is a graphical representation of the hydraulic gradient. Although the gradient is the driving mechanism for horizontal laminar flow, in almost every case there is a vertical component as well. The vertical component of flow may be either in a downward or upward direction in response to recharge or discharge.

Recharge is the addition of water to a ground water reservoir. It is derived from the infiltration of precipitation, interaquifer leakage, inflow from streams and lakes, or, inadvertantly by leakage from lagoons, sewage lines, etc. Discharge occurs where ground water flows to springs, streams, lakes and swamps or is removed by evapotranspiration or wells. Pumping wells have, by far, the greatest impact on the hydraulic gradient, locally steeping it several orders of magnitude.

Ground water velocity is very important in pollution problems because among other things it has a major influence on dispersion. The ground water velocity equation can be derived from Darcy's law and the basic velocity equation of hydraulics. It has the following form:

$$V = KI/a$$

Where a = effective porosity in percent, K = permeability, and I = hydraulic gradient.

Ground water velocities are generally very small, ranging from a few inches per day to a few feet per year.

The openings in a rock are referred to as its porosity, which is expressed as a percent. Porosity determines the maximum amount of water a rock can contain when it is saturated. Porosity can be divided into the part that will drain under the influence of gravity, called specific yield or effective porosity, and the part that is retained as a thin film of water on rock surfaces, which is called specific retention.

CYCLIC FLUCTUATIONS OF GROUND WATER QUALITY

It is generally believed that the quality of ground water changes very slowly, if at all. This may be true in the majority of cases, at least with deep aquifers, those not contaminated, or those not greatly stressed by pumping or construction. On the other hand, many surficial or shallow aquifers are prone to rapid changes in quality, the effect being measurable within days or even hours.

The major cause of cyclic quality fluctuations is the intermittent infiltration of water soluble substances that lie mainly on the land's surface or in the unsaturated zone. These substances may include waste materials, such as brine and chemicals, nitrogeneous materials of natural or man-made origin, and mining wastes to mention only a few of an almost limitless number.

Soluble materials that infiltrate during periods of recharge commonly retain much of their integrity as they migrate to points of discharge, such as wells, streams, springs, lakes, and drainage tiles. Following another period of recharge a second mass of contaminated water may descend to the aquifer and move laterally. In fact, several relatively distinct masses may exist in an aquifer at any instant in time, extending down a flow line and thus resembling a string of beads or a dripping faucet. Furthermore, the contaminated mass may maintain a relatively uniform shape as it migrates through the aquifer.

During the spring runoff, which commonly occurs during

March and April, soil moisture generally exceeds field capacity and massive ground water recharge occurs by means of a wetted front that passes through the entire soil profile. Thus, during the spring one would expect a massive influx of water soluble compounds, such as nitrate, to the ground water system. In many parts of the country the fall season is characterized by decreasing evapotranspiration and temperature coupled with a rainy period. Ground water recharge might occur during this period both by infiltration through macropores and gravity drainage from a saturated soil. Although the quantity of water-soluble materials flushed to the water table in the fall may be areally extensive, the volume of recharge and quantity of soluble salts is likely to be less than would occur in the spring.

During dry weather clay rich soils contract forming fractures of various widths and depths. These fractures, coupled with macropores, may serve as centers of accumulation of salts. Soil adjacent to a fracture becomes drier than the surrounding material and develops a strong capillary potential that pulls soil moisture to the fracture walls, which upon evaporation leaves a lining of water soluble salts. When surface water flows into the openings the lining is dissolved and transported, as a concentrated solution, deeper into the ground perhaps providing recharge and a change in shallow ground quality.

Traditionally we depend on samples collected during a single time interval in order to determine the regional extent of a contaminated area or to evaluate ambient ground water quality, without considering what the samples actually represent. For example, a water sample collected two or three days after a rain might contain a high concentration of nitrate, but if sampled after a 2 to 4 week period of no precipitation, the well water might have a nitrate concentration 1 to 2 orders of magnitude lower. The high concentration represents a contaminated slug of water that was flushed, predominately through macropores from the unsaturated zone to the water table. Moreover, it may represent the maximum concentration that would occur. On the other hand, the less mineralized samples could represent the background concentration. If this hypothesis is correct, water samples should be collected in winter or during summer rainless periods from as many wells as possible during a short time frame. An exception would occur in irrigated areas where it would be more advantageous to sample wells that are pumped continually.

FUNDAMENTALS OF GROUND WATER POLLUTION

In any ground water pollution study it is essential to obtain the background concentration of constituents that might be common both to ground water and a contaminant. Since shallow ground water quality may fluctuate between fairly wide limits during short intervals, it is essential to determine background concentrations by means of several samples taken at different times.

The severity of ground water pollution is related to the characteristics of the waste or leachate, that is, it's volume, composition, concentration of the various constituents, time rate of release of the contaminants, the size of the area from which the contaminates are derived, and the density of leachate, among others. Most of these data are difficult to obtain.

Once a leachate begins to slowly migrate through the unsaturated zone several physical, chemical and biological forces act upon it. Eventually, the leachate may reach saturated strata where it will flow in the direction of the hydraulic gradient. From this point on the leachate will become diluted due to filtration, sorption, chemical processes, microbial degradation, and dispersion. Dilution will continue with time and distance of travel.

Filtration removes suspended particles from the water mass. Dilution brought about by sorption is related to type and amount of clays, metal oxides and hydroxides, and organic matter. Chemical processes are important in attenuation when precipitation occurs as the result of excess quantities of ions in solutions. Chemical processes also include volatilization as well as radioactive decay. In many situations, particularly in the case of synthetic organic compounds, microbiological degradation effects are not well known, but it does appear that a great deal of degradation can occur if appropriate nutrients are available and the system is not overloaded.

Dispersion is a microscopic phenomenon caused by a combination of molecular diffusion, which is important only at very low velocities, and hydrodynamic mixing, which occurs with laminar flow through porous media. In porous media flow paths have various lengths and leachate transport along a shorter flow path would arrive at an end point sooner than that part following a longer path, thus resulting in hydrodynamic dispersion. Dispersion can both be longitudinal and lateral and the net result is a conic form downstream from a continuous pollution source. The concentration of a leachate is least at the margins of the cone and increases towards the source. Since dispersion is directly related to ground water velocity, a plume will tend to become larger with more rapid flow.

The rate of advance of a contaminant plume can be retarded if there is a reaction between its components and the aquifer matrix or native water. The plume, which tends to have a normal distribution, both longitudinally and transversely, will expand more slowly and the concentration will be lower than those of an equivalent nonreactive leachate. Hydrodynamic dispersion affects all solutes equally, while sorption and chemical reactions can affect various constituents at different rates. Thus a leachate plume, consisting of a number of solutes can have each moving at a different rate due to these attenuation processes.

MONITORING WELLS

Not uncommonly monitoring wells are placed in a convenient location that may not be the most appropriate from a hydrogeologic perspective. Moreover, little thought may be given to their design and installation. A monitoring system should be designed and constructed to achieve two purposes: (1) determination of hydraulic gradients and (2) evaluation of quality distribution, both in time and space. Since hydraulic head and ground water quality may vary vertically, horizontally and temporaly, the aquifer should be sampled at a considerable number of times, places and depths.

Traditionally monitoring schemes consist of wells of different depths that are distributed throughout the area of concern but which are generally few in number due to cost considerations. One method is to install a single well and screen it at several different depths. In the first case there may be an insufficient number of wells to describe adequately the vertical and horizontal head distributions, which may be significant in regions of low gradients. In the second case multiple screen wells provide both composite water levels and water samples, which are useful for determining if plume exists but not for locating the actual position and concentration of a plume at depth. In recent years it has become a common practice to install a nest of monitoring wells that are closely spaced but of different depth, lack of which is designed to sample a single interval. The wells may be installed in individual holes or a cluster of wells can be installed in a single, large diameter hole and each screen gravel packed and grouted at a specific depth. Unusual monitoring well designs are appearing with greater regularity in the literature.

The first step in controlling the pollution of ground water, particularly by organic compounds, is the collection of water samples in a manner that will clearly indicate concentrations that actually exist. Although a seemly simple task, the proper collection and storage of water samples for organic analyses is far more complex than most realize and it differs substantially from routine methods used for inorganic compounds.

A common error when sampling for organic compounds is failure to consider the material from which pumps, samplers, discharge lines, storage containers, and well casings are constructed. A typical electrical submersible pump attached to plastic pipe or garden hose, for example, may be entirely inadequate because most of the common metals, rubber, and plastics either contaminate water samples or remove substances from them. Metals may strongly adsorb organic compounds and there is also the possibility of reactions catalyzed by metal surfaces. DDT for example is strongly adsorbed even by stainless steel. Compounding the problem is the presence of lubricants derived from pumps and joints. Many plastics adsorb organic contaminants and bleed other compounds either directly from their surfaces or from the cementing agents used to join pipe segments. Cemented joints should be avoided in any plastic materials used for sampling.

Sampling equipment should be constructed with materials that have the least potential for affecting the sample. Chief among these are glass, which requires careful handling, and Teflon, which is extremely costly. The least desirable material is rubber; unfortunately detailed experimental data on the potential reactions between similar materials and the wide range of organic compounds that serve as real or potential ground water contaminants are simply not available.

In many situations it may be realistic to compromise some accuracy with cost, particularly in regard to materials used for well construction. For example, if the major contaminants are already defined and they do not include any substances that might bleed from PVC or cements used to join PVC, it might be reasonable to use wells cased with this less expensive and more readily obtainable material. Wells constructed of less than optimum materials might be used with a reasonable level of confidence if at least one identically constructed well is available in a nearby uncontaminated part of the aquifer to provide samples for use as blanks. Obviously careful consideration is required in each individual case and the analytical laboratory performing a test should be fully informed of the sampling procedures used and the reasoning behind them.

RESEARCH NEEDS

In order to understand and investigate ground water pollution adequately a number of research projects need to be initiated or examined in more detail. In many cases it is essential that there be cooperative studies in order to have available a wide variety of expertise. It is a truism, although often not admitted, that many of us in special fields tend to work in isolation and as a result depend on old wives tales that we take for scientific principle, or we simply disregard certain aspects because of our ignorance, self confidence, or unwillingness to share a budget that took perhaps weeks or months to obtain.

When considering ground water pollution a broad spectrum of research needs come to light. A few of these include:

(1) Other than a few column studies, some theoretical results, and scanty data obtained from field analysis, very little is known about dispersion in geologic media. Data of these types are essential for predictions and ground water transport models are dependent on them.

(2) Ground water recharge has an impact on water quality. There have been few studies on recharge rates despite the fact that information such as this is fundamental to any hydrogeologic study.

(3) Better ways are needed to evaluate biological, physical and geochemical properties of the subsurface matrix. Usually a small data base is used to evaluate an extremely large, highly complex system. Many of the methods used are primitive at best.

(4) How does the geologic framework act as a control on pollutant migration? We are just beginning to scratch the surface in determining the role of the geologic media, particularly in regard to geochemistry. The geology of an area controls, to a large extent, dispersion and adsorption, both of which attenuate wastes.

(5) Detailed, well instrumented field studies are needed to calibrate both flow and transport models.

(6) Adequate background water quality information is commonly not available, at least with any degree of accuracy. Better methods are needed to evaluate and determine the ambient conditions.

(7) It is becoming increasingly evident that shallow ground water in particular undergoes cyclic fluctuations in quality. A better understanding is needed of the mechanisms involved and their influence on ground water pollution.

(8) How does the vast array of plastics used for monitoring wells and samplers react to water and various contaminants? Do they bleed or adsorb compounds and if so how much and what compounds?

(9) The unsaturated zone can be likened to a vast waste land; no one knows what to do about it or how to go about studying it. Fluid movement through the unsaturated zone can be described by complex mathematics

and models, but from a practical point of view this may well be an exercise in futility. Yet it is the unsaturated zone that has such a strong influence on the migration and attenuation of waste materials that pass through it.

(10) We are only just beginning to examine the role of naturally occurring microorganisms in the subsurface and still little is known of the fate and transport of pathogens.

"The work described in this paper was not funded by the U.S. Environmental Protection Agency. The contents do not necessarily reflect the views of the Agency and no official endorsement should be inferred."

GEOHYDROLOGICAL CONSIDERATIONS: WAYNE PETTYJOHN

TRANSCRIPT OF QUESTIONS/COMMENTS/RESPONSES

Q: Davidson

You said we need to be more concerned with how and when we sample ground water. I wonder why you make this statement if the problems you outlined are indeed occcrring within the unsaturated zone. Should we not be giving more attention to being able to predict, in a spatial sense, what is occuring in the unsaturated zone so that we can predict in advance of reoccurrence what's going to move to the ground water?

A: Pettyjohn:

I think that's very true. I recommend that research continue on the mechanisms of contaminant storage and transport in and through the unsaturated zone. The problem has been that we all work in a dark room. We're very jealous of our ignorance, we don't want people to know how little we do know and we're very jealous of our budgets, which we don't want to share. These are some of the reasons that we constantly choose to work in a closet. The hydrogeologist might never read a report written by a microbiologist or soil scientist; the opposite also holds true. I think that's a sad state of affairs. I wish I knew more about soil science and microbiology.

TRANSPORT AND FATE

PATHOGENS IN WASTEWATERS: TRANSPORT AND FATE OF PARASITES

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ABSTRACT

Animal parasite reproductive stages are regularly found in wastewaters. Some are short-lived while others are long-lived and are capable of surviving the usual wastewater treatment processes. Of all the many parasitic forms that may be present in untreated wastewaters, ascarid and trichurid nematodes and taeneid and echinoccocal cestodes appear to be able to survive aerobic and anaerobic digestion processes. Protozoan cysts are much less resistant to the treatment processes.

In the United States the threat of spread of parasitic disease to man by disposal of digested sludges on land or otherwise, appears to be minor. When presently available technology is utilized efficiently, most parasite reproductive stages can be eliminated from wastewater sludges.

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INTRODUCTION

Sewage is the wastewater of a community. It may be entirely domestic (originating in homes or communities) or it may contain industrial or agricultural wastes. Domestic wastewater is composed of the wastes from human or animal bodies (feces and urine), personal washings, food, laundry, or other procedures used in the cleansing of the home or the community center. It usually contains floating solids such as feces, rags, food scraps, paper, etc., as well as smaller suspended solids such as those that can be flushed down a toilet or be ground up in a food disposal system. It is objectionable in appearance and is considered hazardous because of the pathogens it may contain. Industrial wastewater sometimes contains large particles such as wood chips, rags, plastic containers, etc., as well as dissolved hazardous chemicals in a much higher concentration than does domestic wastewater.

Present day wastes are classed in three general groups; raw sewage and aerobically and anaerobically digested wastewaters. Modern, "developed" societies produce sewage made up of one or more of these wastewaters. Individual homes and small communities usually produce wastewaters which originate from septic tanks or small disposal systems. Somewhat larger communities may produce wastes which are processed through aerobic digestion, then concentrated in sludges and effluents. Larger communities may involve both of the previous systems with a final product being anaerobically digested sewage sludge and effluent. Whatever system is used by the individual or the community, there are certain hazards to health which are of concern.

Waste disposal in "underdeveloped" countries is different and the health hazards are intensified. For example, Pacey (1) reporting on a typical condition in Bangladesh in a town whose sanitation services should have been disposing of 50 tons of human excreta daily, states ...

> "...only 1/10th that amount was adequately dealt with, leaving a balance of more than 45 tons to accumulate in ditches, gutters, roads, and houses. The ground lies thick with human feces, much of them containing intestinal worms which are picked out by the birds. Drainage channels which pass through the town and were intended to convey sullage or storm water, are often almost choked with solids undergoing fermentation. Flies are everywhere and are numerous on the meat and fish that are exposed for sale..."

Our problems in a "developed" society are of far less significance. The risk of contamination with pathogenic microorganisms is reduced because of better developed systems for handling wastes that originate in that society, but the risk of transmission of other pathogens, such as heavy metals or persistent organic chemicals, may be greater.

Transport of Parasites

The extent of transport of parasites in wastewaters is well known from the many reports in the literature. However, introduction and movement of mature parasites or their reproductive stages into a wastewater system is not indicative of a rate of transmission nor is it indicative of the fate of the parasite. Parasitic organisms are not equally resistant to environments outside of the specific hosts. Research has shown that some of them are among the most resistant of microorganisms (2-6). Many can be eliminated from consideration as potential candidates for transmission because of the nature of their life cycles or because of their locations and/or habits in a particular host. The potential for transport and the fate of parasitic organisms in wastewaters is usually dependent upon the following: 1) the type of waste disposal (i.e., raw sewage, aerobic, anaerobically treated, etc.), 2) the level of prevalence of parasitic disease in a community, 3) the degree of severity of individual parasitism, 4) the resistance of the pathogen to the environment, and 5) the intimacy of the potential host with the soil, water, or vegetation which may be contaminated with the wastewater carrying the parasite reproductive stage.

In order to understand the role of wastes in the transmission of parasites, one must consider the variety of life cycles employed by the various parasites. The critical phase, for the parasite, is that involving its transmission from one host to another. In general, there are two types of life cycles. In one, the life cycle is direct and in the other it is indirect, requiring an intermediate host of some kind. With either type of life cycle, the length of time that the parasites survive and their chances of infecting new hosts are the result of a great number of complex factors. The parasite reproductive stage provides an intrinsic factor, the ability of the reproductive stage to resist an unfavorable environment, while the environment itself supplies the extrinsic factors. Parasitic diseases of the blood or other body fluids or tissues, outside the gastrointestinal tract, are unlikely or impossible candidates for direct transmission in wastewaters (7). Also, parasites that require intermediate hosts are not likely to be transmitted in wastewaters, although there may be some exceptions. For example, malaria and filarid worms are delicate parasites in the blood requiring specific environments in the host and, in addition, are transmitted through an intermediate host. The encysted larvae of the nematode worm, *Trichinella spiralis*, are unable to withstand the toxic factors and the temperatures of normal anaerobic digestion longer than 96 hours (8), but probably persist for days in meat scraps in raw sewage. Similarly, sporulated and unsporulated oocysts of the genus *Eimeria* sp. (Protozoa) are killed within 120 hours in anaerobic digesters (9) but may persist in untreated wastes for a longer time. It is assumed that oocysts or encysted stages of *Toxoplasma gondii* and *Sarcocystis* sp., forms closely related to the *Eimeria* sp. but with much thinner oocyst walls, would probably have less resistance to the sewage treatment processes than would *Eimeria* sp.

Persistence of Parasitic Reproductive Stages

The threat of survival and transmission of parasitic organisms from wastewaters is also related to time and to the potential for exposure to man or animals. Figure 1 suggests the situation related to different groups of parasitic organisms. Some forms, such as encysted *Entamoeba* sp., *Giardia* sp., or ova of *Enterobius* sp., which are immediately infective when passed into the wastewater system, may remain infective for only a few hours or days (A). Such forms would normally be a threat to public health in an environment where raw sewage was being dispersed with no further treatment. Other forms, such as the ova of some tapeworms, may be immediately infective for an intermediate host and would be persistently infective in the environment for weeks or months following release into

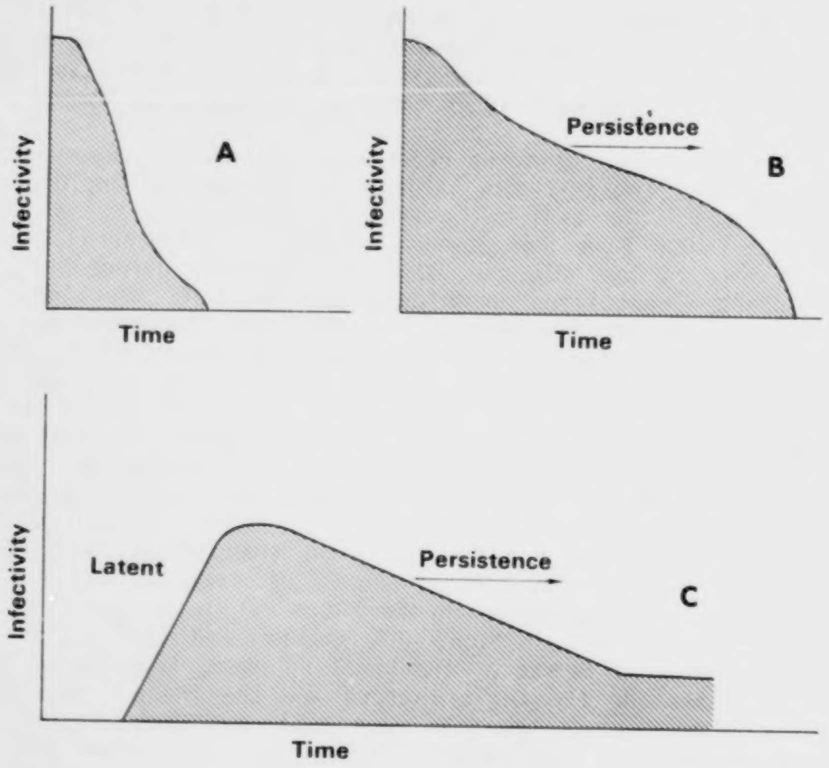

Figure 1. The infectivity and persistence of parasitic infections. A. Parasitic forms (ova, etc.) immediately infective upon passing from the host; survive in the environment a short time. B. Forms immediately infective upon passing from the host and with a moderate survival time in the environment. C. Parasite forms not immediately infective but persisting in the environment and may become infective (adapted from Pacey, 1).

wastewaters (B). Since such forms are somewhat more resistant than those in the first group, they are likely to persist through the first sedimentation and aerobic treatment process. For example, the ova of *Taeniarhynchus saginatus* (the beef tapeworm) may be passed in raw sewage or in aerobic sludges to pastures where grazing cattle may ingest the embryonated ova (10,11). The ovum releases the embryonic tapeworm in the gut and it migrates to the tissues. When the cysticercus in the tissues is ingested by man, an adult tapeworm may develop in the intestine. The third group would include those like the ascarids wherein they are passed into the wastewater in a noninfective state, but persist in the environment, eventually becoming infective for various susceptible hosts (C).

The ability of *Ascaris lumbricoides* ova to resist the various sewage processes has earned it the title of an "indicator" organism for measuring the efficacy of the treatment process. Krasnonos (12) in Russia and Mueller (13) in Germany have reported the survival of the ova of *Ascaris* sp. for 15 and 7 years in soil. Infestation with *Ascaris* sp. worms was and is common in the human population throughout the world. Levine (14) noted that *Ascaris* sp. ova were recovered from a latrine pit in England dating to the 11th or 12th century. Others were identified from the bodies of a 2500-year-old girl and a 1000-year-old man. Ova have been recovered from the surface of dirty bank notes in circulation in Germany. It is particularly significant to know that the ova may pass through the intestines of unusual hosts becoming infective for a second host. For example, Markinkelle and Willems (15) found that the Surinam toad, a common tropical species of the Pacific Colombian coast, ingests feces as well as insects which feed upon feces, then subsequently pass the ova in their feces. Such ova are then capable of embryonating and infesting warm blooded mammals. This points out the potential for spreading the ova in a unique way. In primitive environments this method of spreading the ova could be significant. The ova are known to be resistant to sulfuric, hydrochloric, nitric, and acetic acids, as well as copper and iron sulfates, formalin, chlorine, sodium hydroxide, and other strong chemicals. They show less resistance to some physical stresses such as sunlight, heat, and drying.

Fate of Parasites in Raw Sewage

Parasite reproductive stages (ova or cysts) are usually passed from the gastrointestinal or urogenital tracts of the hosts in feces or urine. Since some of these stages are resistant to the environment, they may survive to invade new hosts. Their ability to survive is essentially dependent upon the physical make-up of the reproductive stage. Those with heavy, thick walls, such as ova from the worms *Taenia* sp., *Ascaris* sp., *Trichuris* sp., etc. and the cysts from the protozoans like *Entamoeba* sp. and *Giardia* sp., are best able to resist a hostile environment. The ova of most arthropods such as the mosquitoes and biting flies do not survive well in raw sewage (16). For this reason, most insects, parasitic upon man or animals, are not likely to be dispersed in raw sewage or in aerobic or anaerobically processed wastewaters. However, if raw sewage is dispersed on land and is allowed to settle in puddles, the resulting clarified water may become a suitable environment for the development of some mosquitoes, etc. Of course, this is an example of a parasite that takes advantage of a created environment favorable to its life cycle.

Each sewage treatment process tends to eliminate some parasitic stages. The usual initial settling of raw sewage in the treatment plant destroys less resistant, thin-walled forms by abraiding, osmotic action or by toxicity. Wang and Dunlop (17) found that primary sedimentation and effluent chlorination reduced the number of *Ascaris lumbricoides* ova in raw sewage by 80%. Vassilkova (18) reported that treatment of raw sewage in Imhoff tanks removed 97% of tapeworm ova. Newton et al. (19) showed that the sedimentation of *Taenia saginata* was time dependent. Two hours settling time was required to remove 98% of the ova from a column of raw sewage. The study by Cram (20) showed that the light cysts of *Entamoeba histolytica* required 3 or more hours to settle to the bottom of a 26-inch high tank. The heavier ova of *Ascaris* sp. settled out in approximately 30 minutes. Other reports have shown that sedimentation removes parasitic stages according to the weight of the organism; heavier ova settling out more quickly than small, lighter ova or cysts. In the treatment process settled material becomes part of aerobic or anaerobically digested sludge.

The outbreak of enteric amoebiasis which occurred at the time of the 1933 World Fair in Chicago, resulting from contamination of drinking water with raw sewage (21), elucidates the ability of some parasites to survive for a time in raw sewage. Attempts to by-pass the wastewater treatment processes in order to utilize raw sewage or raw effluents beneficially have been generally unsatisfactory. The literature is full of accounts of transmission of parasites by spreading improperly treated wastewaters in the environment. The review by Pawlowski and Schultz (3) is one of the most complete dealing with transmission of tapeworms. Shephard (22) has reviewed the problem in developing areas of the world having hot climates where sanitary faciliites are few or nonexistent. The recent reports by Macpherson et al. (11), Sadighian et al. (23), Hammerberg et al. (10), Rickard and Adolph (24), and Arundel and Adolph (34) have reemphasized the significant threat of transmission of tapeworms in raw sewage to livestock. In these instances, the parasitic life cycle is enhanced by the mechanical spread of the ova in raw sewage and sludges to pastures where they are ingested by cattle.

Aerobic Digestion and Secondary Treatment

Most wastewater treatment systems use some kind of additional secondary treatment process in addition to the simple collection of raw wastes and their subsequent initial settling. The Imhoff tank mixes the raw wastes in a specific environment designed to expose them to greater quantities of oxygen and to specific microorganisms which may use the wastes as nutrients. The physical action of the Imhoff tank is that of agitation, and that action aids in abraiding the cell membranes of parasitic organisms. Furthermore, the action of nonpathogenic bacteria may weaken the cell membranes of less resistant parasites. In some instances, the availability of the additional oxygen appears to aid in the hatching process of trichostrongylinate ova and some hookworm ova (20). Reyes et al.

(25) reported that aerobic digestion appeared to stimulate hatching of Ascaris sp. However, such an observation was probably unusual because ascarid ova fall in the category of being noninfective when passed and ova require approximately three weeks to embryonate under ideal conditions (26). Our own studies have shown that the majority of ova that pass through the aerobic and anaerobic treatment processes are unembryonated. Newly hatched larvae of Ascaris sp. are highly susceptible to a harsh environment and they are quickly destroyed. Under normal conditions, the embryonated ova do not hatch until they have been stimulated by secretions, etc., in the gastrointestinal tract.

Primary and secondary sedimentation cause accumulation of heavier parasitic ova such as Ascaris sp. in the sediment. The continuous agitation of aerobic digestion (Imhoff tanks) keeps all parasitic forms suspended until secondary sedimentation occurs. The effluent from aerobic digestion may contain lighter forms such as the cysts of Entamoeba sp. as well as ova, depending upon the characteristics of the aerobic digestion process and the screening and filtering processes which follow. The fate of the parasitic forms is determined by what is done with the raw or aerobically digested sludges. Aerobically digested sludges may contain parasite ova or cysts that are moderately to highly resistant. Hammerberg et al. (10) reported an outbreak of cysticercosis in cattle that were allowed to forage in pastures treated with activated sludge. Eighteen of 40 (45%) cattle slaughtered had cysticerci in the tissues. Of particular significance was the observation that 5 of the 18 (28%) animals infested would have gone undetected by routine carcass examination. Others (27) have made remarkably similar observations about undetected infestations in cattle.

Anaerobically Digested Sludge

A search of the literature fails to reveal reports of specific studies done on septic tank sludges. However, one would assume that, since they are principally the result of anaerobic digestion, the fate of parasitic organisms (ova) would be similar to that in a community anaerobic digester. It is recognized that the biological action would probably be slower because of the lower temperature in a typical septic tank buried in the ground. Mara (28) has pointed out that effluent (or sludge) from septic tanks is as dangerous as raw sewage and so requires adequate treatment before disposal.

For all practical purposes anaerobic digesters are run at temperatures near 35° C. Many parasitic ova, cysts, and other pathogenic microorganisms are destroyed at this temperature, particularly if it is persistent for an appropriate time. Most anaerobic digesters operate on a "continuous feed" basis as contrasted to a "batch" basis. Anaerobic digestion is considered to be complete after 14 days retention in the "batch" digester. Because of the need to handle more wastewaters (sludges) than the system can handle on a "batch" basis, most systems work on a "continuous feed" system wherein 1/14th of the total capacity of the digester is rotated every 24 hours. With this procedure, some sludge is treated for only 24 hours and only a small portion is actually treated for the full 14 days.

For practical purposes, this seems to work reasonably well in breaking down most organic materials, but it is not efficient in destroying pathogens such as Ascaris sp. ova (26). As a result, some pathogens may be distributed to the environment when anaerobically digested sludge is placed on land or is dumped in the oceans. Little information is available as to the fate of parasites (ova, cysts) dumped in the oceans in sludges. Since most of the ova or cysts that reach sludges originate from land animals, including man, those that survive aerobic or anaerobic digestion, are probably not a serious threat to aquatic animals. Regardless, under current laws, ocean dumping in the United States must stop at the end of 1981.

The Effect of Sedimentation and Drying Bed Upon Parasitic Organisms

The basic purpose of the wastewater treatment process is to remove solids. Part of the process is initially done by sedimentation. After some digestive action, additional sedimentation occurs and heavier objects, including parasite ova and cysts, may be accumulated in the sediment (sludge). The majority of the parasites are then present in the raw or digested sludge. Less resistant ova and cysts are unable to withstand the concentration effect brought about by the sedimentation. However. ova like Ascaris sp. are very resistent to materials present in the sludge. They are the most resistant of the parasitic organisms in wastewaters and are resistant to the usual wastewater treatment plant processes. Sedimentation only concentrates the ova in sludges and neither aerobic nor anaerobic digestion destroys all of them. Exposure to chlorine at the concentrations usually used in effluents has little, if any, effect on them. However, sodium hypochlorite (chlorox) in concentrations greater than 5% dissolves the outer shell when contact is greater than 30 minutes. With the outer shell removed the ovum is more susceptible to environmental conditions.

Ascaris sp. ova are able to survive in an unembryonated condition for several years in sludge yet remain capable of embryonating when conditions are favorable (26). As a matter-of-fact, they may be less susceptible to a harmful environment while they are unembryonated. Storage of liquid sludge in a lagoon also fails to destroy reproductive forms such as Ascaris sp. ova. We have found that some Ascaris sp. ova, recovered from lagooned sludge that had been stored for many years, were capable of embryonating. Reimers et al. (29) reported that they found Ascaris sp. and Toxocara sp. ova were destroyed when the moisture content of sludge was 20%. Most drying beds do not obtain a moisture content that low. More frequently the moisture content would be in the range of 70 to 80% moisture. Some ova are apparently able to withstand the dehydration process in the drying bed followed by prolonged storage in dried sludge such as Nu-earth. There are few reports which give details about the rate of "die-off" of parasite ova, because no one has carried out a study long enough to determine an end point. Current studies at the University of Cincinnati may supply the answer.

The Effect of Composting Sludges

Pathogenic organisms in wastewaters are variously affected by the digestion processes to which they are exposed when passing through a sewage treatment plant. Some pathogens are inactivated by the aerobic and anaerobic processes; others may be reduced in number by exposure to chemicals such as chlorine that is added to effluent, and a few are reduced in number by several years storage in lagoons. Chemical treatment of sludges may be useful in reducing the numbers of some kinds of pathogens but others, like Ascaris sp. ova, are not affected by most chemicals that are present in sludges or that may be added to control the pathogens. Irradiation of sludges with radioactive materials destroys pathogens, when used at appropriate levels, but other restrictions on its use such as cost, time, handling, equipment, etc., make its use somewhat questionable. A temperature-time treatment of sludge is the most appealing at present, if a significant need to control pathogens in sewage sludges is demonstrated. Poincelot (30) has pointed out that as a composting system proceeds with aerobic digestion, the various microorganisms (nonpathogens) use carbon, nitrogen, and other nutrient elements in their growth patterns. As their life cycles proceed the temperature goes up, down, or remains constant depending upon the biological activity. In composting sludges, the generated heat is retained in the compost pile. Wiley and Westerberg (31) and others have shown that temperatures in the range of 60-70° C effectively destroy the pathogens which are usually considered harmful to man and other animals. For example, Ascaris sp. ova are killed when subjected to 60° C for 30 minutes. If the compost pile is properly constructed and maintained, the internal temperature of the pile may reach 60+ C and the protozoan and helminth parasites will be inactivated. However, if the pile is neglected and the external surfaces, which often remain cooler, are not turned into the pile, the resistant ova of Ascaris sp. and others may survive the composting process (30). Windrow composting is usually carried out for 18 to 21 days at a temperature above 55° C. If this time-temperature element is completed, the pathogens will be eliminated except for some fungi which are able to withstand higher temperatures (32).

Organisms in Wastewaters

In studies designed to determine the survival and occurrence of parasitic and other organisms present in raw sewage, anaerobically digested sludge and effluent, we examined samples obtained from five treatment plants of the Metropolitan Sanitary District of Greater Chicago, for a period of 15 months. All parasitic and free-living organisms were identified and the number per liter and in 100 gm dry weight samples were calculated (16). Tables 1, 2, and 3 are listings of the parasitic and free-living organisms recovered by biweekly samplings of five sewage treatment plants. Table 1 shows the mean number and kinds of organisms found in raw sewage entering each of the five plants including the West-Southwest (the worlds largest) plant. Table 2 shows the occurrence of organisms that were identifiable in "draw-off, grab" samples from anaerobically digested sludge taken from the digesters from four of the plants. In all cases, adults and larvae of different forms were dead, but some of the parasite ova were determined to be viable. In an earlier study, (26) it was determined that approximately 77% of Ascaris l. suum ova, held in anaerobic digesters at 38° C for 25 days, were capable of embryonating when placed under "embryonating" conditions. Table 3 shows the organisms present in effluent. It suggests that when waste disposal systems function properly few parasitic organisms reach the environment in wastewater effluent. However, there are large numbers of free-living active forms present which an untrained observer may confuse with pathogenic parasitic forms. We found that parasitic ascarid, trichurid, and cestode ova and coccidian oocysts and amoebic cysts were recoverable from raw sewage and anaerobically digested sludge but almost all were eliminated from effluent. In contrast, there were great numbers of other invertebrates present in all three types of samples although most in the anaerobically digested sludge were dead.

Natural Transmission of Parasites

Hays (3) and Fitzgerald (10) recently reviewed the potential for transmission of parasitic disease by land application of sewage plant effluents and sludges. However, potential and actual transmission of pathogens are entirely different situations. For example, the accumulation of raw wastes in the streets and ditches of Bangladesh would be far more likely to produce disease caused by microorganisms than would properly digested wastewater sludges, produced in a modern sewage treatment plant, which was spread on farm land.

In an experiment designed to determine whether Ascaris sp. ova naturally present in Anaerobically Digested Sludge (ADS), were capable of embryonating and infesting pigs, three young Specific Pathogen Free (SPF) pigs were confined in "closed" facilities and were each given a total of 12 liters of liquid sludge (400 ml/day) containing about 600 ova, during a period of 30 days. The AD sludge had previously been aerated by agitation and bubbling air through it for 104 days. Seventythree days after they first received sludge in the diet, the pigs were necropsied and the livers, lungs, and intestines were examined. One of three had ascarids in the intestine, but no larval stages were recovered from the lung or livers.

In studies involving more than 178 SPF pigs, it was determined that natural transmission of Ascaris sp. from anaerobically digested sludge is possible. Liquid or air dried anaerobically digested sludge was placed in pens at various levels from the equivalent of 20 to 1134 dry metric tons per hectare. Pigs were then confined in the pens and were allowed to live naturally for four months in close association with the sludge. At necropsy some of the pigs had larval or adult worms in the intestines. In a few, active larvae were recovered from the liver and lungs. These results indicate that some ova from Ascaris sp. are able to survive the treatment process and may infest animals naturally. With ova present at up to 4000 per square meter of surface, embryonation of some ova occurred, were ingested by pigs, and eventually caused light infestations in the gastrointestinal tracts. The exposure of the swine to anaerobically digested sludge was infinitely greater than a normal human exposure would be, yet, the levels of infestation with Ascaris sp. were low (33).

Table 1. Parasitic and free-living organisms isolated by sucrose flotation from raw sewage collected biweekly (February 1976-May 1977) at five sewage treatment plants of the Metropolitan Sanitary District of Greater Chicago.

	Mean number of organisms/100 grams of solids (number of samples)				
Organisms	Lemont (28)	West-Southwest (27)	Hanover Park (28)	Calumet (27)	Northside (27)
			Parasitic Forms		
Ascaris sp. ova	0	29	78	0	29
Toxocara sp. ova	246	86	90	79	232
Toxascaris leonina ova	0	21	0	0	93
Enterobius vermicularis ova	109	0	0	0	0
Cestode ova	0	22	0	0	0
Coccidian oocysts	4	68	0	0	20
Entamoeba coli cysts	0	5	0	0	0
			Free-living Forms		
Nematode ova	4889	1531	1316	1935	3236
Nematode adults	4735	3139	3983	2473	4415
Nematode larvae	5516	4540	5322	3296	7712
Mite ova	6059	2825	2408	1644	3574
Mites (parts)	908	198	202	269	315
Dipteran ova	122	0	31	35	20
Dipteran larvae	0	22	29	6	12
Rotifers	23439	725	1927	1171	791
Cladocera	0	15	369	569	63
Copepods	0	22	0	98	0
Tardigrada	15	11	0	0	0

Other studies by our laboratory have shown that cattle exposed to anaerobically digested sludge that was variously dispensed on forage and land failed to show evidence of increased parasitism. The ADS used during this study contained the levels of organisms shown in table 2 and was dispensed during the summer months for seven years. Necropsies of 71 adult cows, 123 yearling calves and 16 fetal calves failed to show any tissue parasites and there was no significant difference in the number and kinds of intestinal parasites in control and experimental animals. Table 2 suggests that the ova that were present in the ADS, originating from the MSDGC system, were potentially transmissible to cattle but if they were viable and were transmitted they failed to develop to a detectable stage. Studies

Table 2. Parasitic and free-living organisms isolated by sucrose flotation from anaerobically digested sludge collected biweekly (February 1976-May 1977) at four sewage treatment plants of the Metropolitan Sanitary District of Greater Chicago.

	Mean number of organisms/100 grams of solids (number of samples)			
Organisms	Lemont (30)	West-Southwest (29)	Hanover Park (30)	Calumet (29)
		Parasitic Forms		
Ascaris sp. ova	48	144	232	14
Toxocara sp. ova	440	153	283	157
Toxascaris leonina ova	0	18	0	13
Trichuris sp. ova	6	28	9	11
Enterobius vermicularis	0	0	0	0
Cestode ova	0	53	0	0
Coccidian oocysts	94	268	17	21
Entamoeba coli cysts	0	14	0	0
		Free-living Forms		
Nematode ova	4311	996	2363	2476
Nematode adults	1134	74	159	425
Nematode larvae	1013	111	314	408
Mite ova	26558	4154	6087	4345
Dipteran ova	109	24	32	50
Dipteran larvae	0	7	0	8
Rotifers	1355	299	434	396
Cladocera	11	44	23	152
Copepods	0	0	7	13
Tardigrada	15	0	0	0

by others have shown that cattle may become infested with larval tapeworms when allowed to forage in pastures treated with raw sewage (10,24,34). Various researchers have suggested that pastures or other forage should not be fed to cattle until a significant time has elapsed after treatment with sludges. In our studies no specific time was allowed before animals were allowed to graze sludge-treated forage. In some instances, cattle were allowed in the pastures at the same time the anaerobically digested sludge was being applied by overhead irrigation. Obviously, the kind of sludge being dispersed is critical, but it would appear that in some instances the so-called "waiting period" is grossly overemphasized.

Table 3. Parasitic and free-living organisms isolated by sucrose flotation from unchlorinated effluent collected biweekly (February 1976-May 1977) at five sewage treatment plants of the Metropolitan Sanitary District of Greater Chicago.

	Mean number of organisms/100 grams of solids (number of samples)				
Organisms	Lemont (28)	West-Southwest (27)	Hanover Park (28)	Calumet (27)	Northside (27)
			Parasitic Forms		
Ascaris sp. ova	0	0	0	0	0
Toxocara sp. ova	0	0	0	0	0
Toxascaris leonina ova	0	0	0	0	0
Trichuris sp. ova	0	154	0	0	0
Enterobius vermicularis ova	0	0	0	0	0
Cestode ova	0	0	0	0	0
Coccidian oocysts	0	0	0	0	220
Entamoeba coli cysts	0	0	0	0	0
			Free-living Forms		
Nematode ova	705	81744	5752	1087	11826
Nematode adults	1212	55352	15402	10060	67152
Nematode larvae	1422	70116	23391	9945	82733
Mite ova	611	926	2613	111	1533
Mites	597	637	426	784	1098
Dipteran ova	0	135	216	0	0
Dipteran larvae	1024	0	38	132	212
Rotifers	116057	42017	4384	2217	10528
Cladocera	83	0	0	148	0
Copepods	461	0	294	6142	0
Tardigrada	0	0	82	0	119

DISCUSSION

In recent years the people of the United States have become more acutely aware of the environment in which they live. They are more concerned with the air they breath, the water they drink, the food they consume, and the industrial contaminants to which they are exposed. The Federal and State governments have placed increasing numbers of restrictions and controls on specific items of environmental concern, among them are the methods by which wastes are disposed. In referring to primitive conditions in China nearly 60 years ago, Faust (35), indicated that by far the largest portion of diseases were caused by communicable organisms which were voided by man in fecal excreta. Parasitic diseases were common because of the lack of knowledge about disease transmission and life cycles of the various parasites. During the same period parasitic diseases were also a significant problem in the United States. Over the years since 1924, the United States has nearly doubled its population but, because of concern for the health of the individual, it has greatly reduced the diseases caused by parasitic organisms. Parasitic diseases of humans are now a minor problem in the United States. Only a few human medical schools give significant course work dealing with human parasites. However, parasites of animals remain a significant threat to the health of animals, and in some cases, to man.

The present trend of disposing of waste sludges by spreading them in or on the soil brings a new dimension to our traditional procedures. With the recent influx of humans from areas of the world where parasitic diseases are common, we have increased the potential for the spread of parasitic disease. Fortunately, many of the introduced parasitic diseases are not a threat to the general population because of various environmental restrictions which naturally limit the spread of the organisms. However, as has been pointed out, a few parasites are capable of surviving the more commonly employed sewage treatment processes. It would appear, however, that parasitic organisms that survive the treatment processes are not significant or life threatening diseases and could be transmitted to man only under unique or unusual circumstances.

Unfortunately, we cannot use man as an experimental subject in most of our research work, so we find substitutes and then, by "educated guesses", try to extrapolate the findings of animal experimentation to man. Examples of such studies were noted with the exposure of Specific Pathogen Free pigs to wastes from a large cosmopolitan society. Under conditions of extreme exposure, some parasitic organisms were shown to survive in wastes for a considerable time and to be transmissible to swine. If man was subjected to a similar "extreme" exposure, it is conceivable that he could also become infected with specific kinds of parasites.

All parasitic protozoa, helminth, and arthropod reproductive stages can be inactivated by appropriate heating, drying, or by exposure to specific levels of radiation. At present, the least expensive, effective method of complete inactivation is that of composting wastewater sludges. Prolonged heating above 60° C inactivates animal parasite reproductive stages. Spreading sludges on or in the soil aids in drying the sludges but does not inactivate all ova as shown by Mueller (13) and Fitzgerald (33). Sludges taken from the drying beds are not usually dry enough to affect all of the ova in the sludge. Irradiation may effectively inactivate all pathogens in sludges but the cost effectiveness would appear to be an imposing barrier to its practical use.

The results of research discussed herein and in the several reviews such as those of Hays (2), Keller (5), Rudolfs (6), Shephard (22), and

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Toxascaris leonina ova	0	0	0	0	0
Trichuris sp. ova	0	154	0	0	0
Enterobius vermicularis ova	0	0	0	0	0
Cestode ova	0	0	0	0	0
Coccidian oocysts	0	0	0	0	220
Entamoeba coli cysts	0	0	0	0	0
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Cladocera	83	0	0	148	0
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DISCUSSION

In recent years the people of the United States have become more acutely aware of the environment in which they live. They are more concerned with the air they breath, the water they drink, the food they consume, and the industrial contaminants to which they are exposed. The Federal and State governments have placed increasing numbers of restrictions and controls on specific items of environmental concern, among them others have identified the parasitic organisms that are potentially transmissible to man. The fate of the parasite is dependent upon its ability to resist the environment and, secondly, upon the opportunity for ingestion, by the host, of the reproductive stages. In the United States, other methods of transmission do not appear to be significant.

Of all the parasitic organisms, only the ascarids and trichurids of the nematodes and the taeneids and echinococcal cestodes appear to be significantly resistant to modern day aerobic and anaerobic treatment processes. Reproductive stages such as cysts of *Entamoeba* sp. and *Giardia* sp. are, for the most part, not resistant to the processes. Other parasitic forms may be ruled out because they require intermediate hosts not readily available. Research has shown that viable ova of *Ascaris* sp. may persist in sewage sludges for years. No other parasitic organism is known to persist outside a host for such a long time.

In summation, it would appear that the fate of most reproductive stages of animal parasites in wastewater depends upon how the wastes are handled. If wastewater treatment processes are carried out properly, most parasites will be inactivated. The few that survive may be a health hazard under conditions where there is a possibility of ingesting or breathing in soil containing reproductive stages. In the United States, this does not constitute a significant threat to man, but it may be a source of infection to animals. In spite of the technology for wastewater treatment which presently exists in the United States some sludges and wastewaters areimproperly treated. As indicated earlier, the potential for transmission of parasitic diseases rests upon the make up of the wastes and the exposure of the host to the wastes. The parasites which are most likely to survive specialized treatment processes are limited to a few nematodes and cestodes. Since these do not multiply in the body of the host like viruses, bacteria, and protozoans, it is likely that low levels of infestation, which might originate from ingestion of reproductive stages present in treated wastes, would be of minor concern. Present day available methodology appears to be adequate to effectively inactivate parasitic organisms and to control parasitic disease transmission in wastewaters.

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TRANSPORT AND FATE - PARASITES: PAUL FITZGERALD

TRANSCRIPT OF QUESTIONS/COMMENTS/RESPONSES

Q: Gregg

Did you test the ground sludge that the pigs lived in for the four or five months to see what organisms might still have been there after that period of time?

A: Fitzgerald

Yes. This particular experiment that I showed was broken out of raw mineral soil. It had been strip mined and nothing had been grown on it. It had never had any kind of animals on it. This was part of the reason we chose it because there wouldn't be any contamination. I might point out that we did do another experiment in which sewage sludge was applied to similar kinds of soil, but it was applied during the previous five years and then incorporated into the soil. The last application was made one year prior to the time that we put SPF pigs on that particular soil. Out of seventeen pigs that we put there, three of them became infested with ascarids, which indicates to me that the ascarids persisted in that they were present in the sludge and that they persisted in the soil for a long period of time. I don't know whether that was five years or one year, but I know that they persisted at least one year and they were infective to the animals at that time.

Q: Kreissl

It wasn't completely clear to me what you considered the fate of parasites in septic tanks soil disposal systems. You've talked a lot about the major type treatment facilities, the urban kind of facilities, but __________.

A: Fitzgerald

Someone has said that septic tank sludge is just as dangerous as raw sewage and should be treated essentially the same as raw sewage. Now, it would depend upon several factors as to what the fate of the parasite is; this was my point in trying to bring out the significance of the level of parasitism in a particular environment, and what the level of parasitism was in an individual person. That would indicate what was in the septic tank since the septic tank is a closed system. It's not generally for a large community; it's for a family or perhaps two families, and this would indicate that parasites may or may not persist. Essentially, there is no work being done with septic tanks as such. A septic tank system is an anaerobic system (on which a lot of work has been done), but not with septic tanks alone.

TRANSPORT AND FATE - BACTERIAL PATHOGENS IN SOIL

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ABSTRACT

Application of domestic wastewaters to soil presents potential public health problems. Sewage likely contains many pathogenic bacteria with Salmonella probably the most prevalent. The survival of salmonellae in soil and their tendency to leach through soil was investigated under controlled conditions in the laboratory. The time required for 99.9% reduction in population was dependent on soil, incubation temperature and soil moisture. Significant interactions occurred between factors. Soil temperature above 35°C was consistently detrimental to salmonellae survival and generally less than 2 weeks were required for 99% reduction in population. Dry soil was not as suitable for salmonellae survival as moist soil. The salmonella population actually increased before it began to decline when moist soil incubated at 22°C was inoculated. Leachability of salmonellae through soil was dependent on the soil. Soils containing more than 35% clay were able to reduce the populations of salmonellae by 99.9% by passage through only 5 cm of soil. By contrast a sandy soil reduced the population by 99%. The combined effects of poor survival of salmonellae in soil, adsorption, and filtration greatly reduced the likelihood of salmonellae reaching groundwaters when normal irrigation practices are followed. Research is needed to establish a guideline on permissible levels of salmonellae that may be added to soil without significant potential for endangering public health. It does not seem reasonable or prudent to require domestic wastewaters be completely free of bacterial pathogens before land application.

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TRANSPORT AND FATE - BACTERIAL PATHOGENS IN SOIL[1]

R. W. Weaver[2]

INTRODUCTION

People are very interested and concerned about the health hazards of disposing or utilizing wastes by land application because any disease organisms in the human or animal population generating the wastes would likely be present in the wastes. The fate of pathogenic organisms in soil must be known to ascertain the potential threats to public and animal health. Excellent review articles have already been prepared on this topic by Rudolfs et. al. 1950; Mallmann, 1951; Krone, 1968, Dunlop, 1968; and Bernarde, 1973. Another review article would not likely make much of a contribution. Instead it is my purpose to provide results of research conducted in my laboratory that has a bearing on this topic and may serve to advance the knowledge regarding the fate of salmonellae in soil. The experiments reported in this manuscript were conducted by three graduate students I supervised, and constituted part of their thesis research (Zibilskie, 1975; Fehrmann, 1977 and Webb, 1978). For brevity only the salient aspects of their research has been included.

Salmonellae were chosen as the model bacterial pathogen to conduct research on because it appears to be prevalent in animal and human wastes and there has been considerable interest in its survival in soil. Several selected references on this topic have been cited in the reference section of this report. The contribution of each paper will not be summarized here but they will be considered in a general way as regards the current state of knowledge about salmonella survival in soil.

Salmonellae occur widely in nature but enrichment methods are needed for their detection (Thomason et. al., 1977). Most investigations regarding survival of salmonellae in soil have been conducted by artificially inoculating the soil and determining how long the organism could be detected. Most field experiments have been conducted with little control over environmental conditions. The results of such investigations indicated that salmonellae may survive a time period from a few hours in hot dry soils to more than 2 months.

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The main criticism of the field research is that the conditions have not been well enough defined for development of principles regarding limiting factors of salmonellae survival in soil.

I am not aware of specific research on movement or leaching of salmonellae in soil. Other motile bacteria do not actively move more than a few centimeters in soil over a period of several days unless the moisture content of soil is at or near field capacity (Hamdi, 1971). The limitation to movement is the tortuous pathway to be followed to obtain continuous pores having water films of adequate thickness for the flagella to be functional. The principles involved regarding movement and leaching of bacteria in soils have been thoroughly discussed by Krone (1968). It appears that active movement by bacteria in soil is not of significance in their transport but passive movement by the leaching action of water is the main transport mechanism.

MATERIALS AND METHODS

SURVIVAL OF SALMONELLAE IN SOIL

Cultures and Inoculant Preparation

The organism utilized in Experiment I was a clinical isolate obtained from Dr. B.G. Foster, Biology Dept., Texas A&M University. In Experiment II salmonellae isolated from feedlot cattle and isolates obtained from Texas A&M University College of Veterinary Medicine were utilized in all investigations. Additionally a second salmonella isolate from feedlot cattle and the clinical isolate from Experiment I were included in an investigation to determine differences in survival of isolates. All cultures were confirmed as *Salmonella typhimurium* by biochemical tests and serotyping.

Inoculants were prepared from 24 hour dulcitol broth cultures grown in an incubator shaker at 37°C. The broth consisted of 2.5g dulcitol (Difco), 2.0g yeast extract (Difco), 5.0g peptone (Difco), 1.0g K_2HPO_4 and 0.005g brilliant green per liter of distilled water. The cultures were centrifuged and washed three times with 0.42% NaCl in water before inoculation of soil or manure.

Manure served as the carrier of salmonellae in some investigations. Fresh manure was obtained from beef-cattle and was examined for the presence of salmonellae by adding 1g subsamples to tetrathionate broth (Difco) and culturing for 12 hours at 37°C. After enrichment, cultures were streaked on brilliant green agar and MacConkey agar (Difco) for salmonella isolation. Manure not containing salmonellae was slowly dried at room temperature to 20% solids content on a dry weight basis (computed by dividing the dry weight of solids by the weight of water). The manure was inoculated by adding 1 ml of culture to 10g of manure slurry. The population of salmonellae in the manure ranged between approximately 1 x 10^6 and 1 x 10^8 per g depending on the population density desired for the particular investigation.

Experimental units

Wide mouth glass vials of 25 ml capacity were used for the investigations. Snap on plastic lids having two 1 mm dia. holes were used to reduce evaporative loss of water and provide for aeration. Each vial contained 10g of soil (dry weight basis) previously adjusted to the appropriate moisture tension. A pressure plate extractor which forced excess water from the soil with air pressure was used to adjust soils to -0.5 atm. moisture tension. For saturated soil conditions water was added to the vials to a height of 0.5 cm above the level of soil.

Inoculation of soil

Two methods of inoculation were used in each experiment. In Experiment I inoculum was added with manure slurry or with saline. Soil in each vial received 0.5g of manure slurry or 0.5 ml of inoculated 0.85% saline solution. The inoculum was throughly mixed into the soils.

In Experiment II inoculum was only added with manure as the carrier. Manure slurry was added at a rate of 0.15g per vial. According to the treatment, manure was left on the soil surface or incorporated by mixing.

Enumeration media and procedure

A medium developed for use in Experiment I had the following composition; 2.5g dulcitol, 2g yeast extract (Difco), 5g peptone (Difco), 5g sodium thiosulfate, 0.25g ferrous sulfate, 0.50 ferric ammonium citrate, 1.0g K_2HPO_4, 0.010g brilliant green (Difco), and 15g agar (Difco) per liter of distilled water. Brilliant green was added aseptically after combining and autoclaving the other ingredients. Before autoclaving but after dissolving the ingredients by closely monitored heating, the pH of the medium was adjusted to pH 6.9 with 0.1 N HCl. Brilliant green was sterilized by filtration and was added to the medium after it cooled to 50°C.

Commercial media; salmonella-shigella agar (Difco), brilliant green agar (Difco), MacConkey agar (Difco) and bismuth sulfite agar (Difco) were not selective enough for use as enumeration media. A variant of the brilliant green medium developed by Moates and Kinner (1976) was utilized in Experiment II because the salmonella isolates used in this experiment would not grow satisfactorily on the medium used in Experiment I. The medium consisted of 40g trypic soy agar (Difco), 10g dulcitol, 0.08g phenol red, 2.0g sulfanilamide (U.S. Biochemicals), 1.6g ferric ammonium citrate, 5.0g sodium thiosulfate, 5.3g l-lysine monohydrochloride (Calbiochem) and 0.006g brilliant green per liter of distilled water.

The entire contents of the vials were used at each enumeration. For Experiment I, contents were transferred to 500 ml flasks containing 100 or 50 ml of sterile distilled water respectively for the first three samplings and the next three samplings. The flasks were shaken by hand for 15 seconds. Serial dilutions were made using saline blanks. Spread plating using 0.1 ml of inoculum provided a lower limit of detection of 100 or 50 salmonellae per g soil depending on sampling. An enrichment broth using 5 ml of the original dilution was used to increase the lower unit of detection to 2 or 1 salmonella per g soil. Tetrathionate broth was streaked for salmonella isolation after 18 hours incubation at 37°C. Representative suspect colonies were confirmed as Salmonella by biochemical tests and serotyping. Plate counts were made after 3 days incubation at 37°C to allow time for black centered colonies to form due to H_2S production.

For Experiment II a slightly different procedure was followed. The contents of the vials were transferred to 100 ml of half strength physiological saline and dispersed in a blender for 60 seconds. A dilution series was made using half strength saline and 0.1 ml of inoculum was used for spread plating. Enrichments using tetrathionate broth were made as in Experiment I. The detection limit for Experiment II was the same as for the first three samplings of Experiment I. Plates were incubated for 24 hours at 37°C before counting colonies.

Soils

Two soils were used in Experiment I and two additional soils were included in Experiment II. Some characteristics of the four soils are listed in Table 1. The soils were selected to provide a range of pH, organic matter content and clay content. Soils are classified as follows: 1. The Amarillo sandy loam is a fine-loamy, mixed thermic Aridic Paleustalf, 2. The Bowie sandy loam is a fine-loamy, siliceous, thermic Fragic Paleudult, 3. The Houston Black clay is a fine, montmorillonitic, thermic Udic Pellustert, 4. The Beaumont clay is a fine, montmorillonitic, thermic Entic Pelludert. Soils were collected from the surface horizon, air dried, passed through a 2 mm sieve and thoroughly mixed.

Experimental design

Experiment I: A factorial experiment without replication was chosen so that five factors could be examined for their affect on survival of Salmonella. The factors were soil, incubation temperature, moisture content, inoculum carrier and time. Levels of each factor were respectively, (1) Houston Black clay and Amarillo sandy loam; (2) 5, 22 and 37°C; (3) 0, -0.5 and $<$ -22 atm. moisture tension; (4) inoculum carriers saline and manure; (5) sampling times 0, 3, 7, 21, 42, 63 and 84 days.

Experiment II: A factorial experiment with three replications and three factors were used to determine time required for 99.9% reduction

Table 1. Physical and chemical properties of soils used in investigations on survival of salmonellae.

Property	Amarillo sandy loam	Bowie sandy loam	Houston Black clay	Beaumont clay
Texture				
% sand	59	76	13	20
% silt	13	11	36	30
% clay	28	13	51	50
pH	7.7	5.1	7.5	5.5
Organic matter (%)	0.8	0.6	3.1	2.4
Pw (-0.5 bar)	12	8	23	26

in the population of salmonellae. The factors were soil, method of manure incorporation and incubation temperature. Levels of each factor were respectively, (1) Houston Black clay, Amarillo sandy loam, Bowie sandy loam and Beaumont clay; (2) manure added to the soil surface or incorporated by mixing, and (3) 5, 15, 5-15 diurnal variation, 35 and 15-35°C diurnal variation. Samplings were made about every 2 days of incubation early in the experiment but later sampling times increased to approximately 5 days between samplings. Survival curves were plotted to determine time required for 99.9% reduction in salmonella populations.

LEACHING OF SALMONELLAE THROUGH SOILS

Soil

Three soils having a range of physical and chemical characteristics were utilized in these investigations. Some properties of the soils are indicated in Table 2 and Figure 1. The classification for the Houston Black clay was provided on the previous page. The Arenosa loamy sand is a thermic, coated, Typic Quartzipsamments and the Angelo sandy clay loam is a fine, mixed, thermic, Torrertic Calciustolls.

Cultures and media

Two Salmonella species were included for comparative purposes. A clinical isolate of S. typhimurium (also used in Experiment I) and an isolate of S. enteriditis made from the San Angelo sewage farm were used in the investigations. Late log phase nutrient broth cultures were utilized for inoculations. Brilliant green agar (Difco) was used for spread plating serial dilutions of soils and soil leachates. Physiological saline was the diluent.

Columns for leaching

The columns consisted of modified Millipore Swinnex 25 mm filtering assemblies having a 22 mm diameter pyrex tube inserted into the top portion. A 3 um Nucleopore filter was used on top of the filter unit to retain the soil. This filter allowed bacteria to pass through without a reduction in population.

Soil column heights of 1, 2, 3, 4, 5, 10 and 15 cm were prepared by sequentially adding 5g of air dry soil and tamping until the desired column height was obtained. A constant soil bulk density in the columns was achieved by using a number 1 rubber stopper attached to a glass rod as a tamping tool. Tamping was accomplished by dropping the tool ten times from a height of 15 cm above the soil surface after each 5g of soil was added.

Addition of inoculum to columns

Inoculum was carefully poured into the columns in a manner that minimized disturbance to the soil surface. For each cm of column height

Table 2. Physical and chemical characteristics of soils used in investigations on adsorption and leaching of salmonella.

	Soil		
Property	Arenosa loamy sand	Angelo sandy clay loam	Houston Black clay
Texture			
% Sand	84	47	11
% Silt	6	18	35
% Clay	10	35	54
pH	4.9	7.3	7.5
% Organic matter	1.3	3.6	3.1
Hydraulic conductivity (cm/hr)	5.81	0.61	0.16
% Pore space	53	58	52

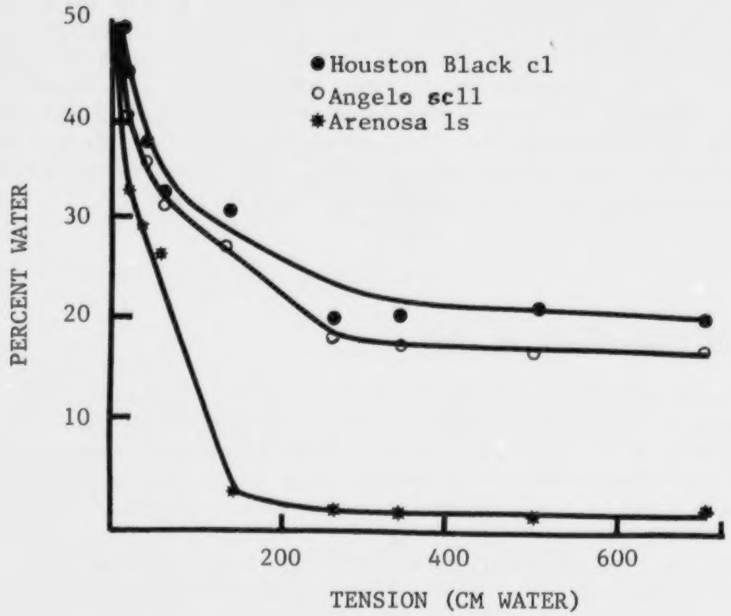

Figure 1. Soil moisture content of three soils, used in leaching experiments, as a function of moisture tension.

5 ml of inoculum having an approximate cell density of 1 x 10^6 salmonellae per ml was used. Physiological saline was the diluent. After addition of the appropriate amount of liquid it was allowed to percolate through the column under the force of gravity and capillary action until the wetting front reached the bottom. A vacuum of 0.8 to 0.9 atmospheres was then applied until drainage ceased. The leachate was collected and the salmonellae enumerated. The entire process required less than 1 hour. Approximately 80% of the added liquid passed through the columns.

Adsorption of salmonella to soil particles

Inoculum was added to 25g of air dry soil in 250 ml centrifuge tubes. The quantity of inoculum added (25 ml) completely saturated the soil. After standing for 5 minutes an additional 220 ml of saline was added and the mixture was shaken for 5 minutes on a mechanical shaker. The tubes were centrifuged at 1500 rpm for 3.5 minutes to sediment soil particles 1 um in diameter or larger. The liquid was decanted and the procedure repeated to reduce the likelihood of significant numbers of salmonellae being carried to the bottom of the tubes by entrapment with soil particles. The liquid was decanted and combined with the other liquid and salmonellae were enumerated by plating.

RESULTS

SURVIVAL OF SALMONELLAE IN SOIL

The main effects of Experiment I cannot be examined in a meaningful manner because there were significant interactions between several factors (Table 3). For brevity only some treatment results will be presented and discussed. All of the data have been previously presented by Zibilskie (1975) and Zibilskie and Weaver, (1978).

Generally the temperature of 39°C was very detrimental to salmonellae survival. In no case was salmonella detected beyond 3 days when the treatment combination was 39°C and <-22 atm. moisture tension. Other treatment affects were not consistent due to interactions. An example of a soil and moisture interaction occurring at 39°C is indicated in Table 4. When soil moisture was high (-0.5 atm. tension), in the Amarillo soil, salmonellae were detected after 21 days incubation at 39°C but for the Houston Black, salmonellae were not detected beyond 3 days.

Salmonellae incubated at 22°C also showed a range in survival dependent on soil and moisture treatments (Table 5). Generally, the survival was longer than that noted for 39°C but for the treatment combination of Houston Black and low soil moisture salmonellae were not detected 3 days after inoculation. For other treatment combinations with the incubation temperature of 22°C salmonellae could still be detected at 42 days. The population actually increased during the first 3 days

Table 3. Significant interactions that occurred in Experiment I involving *S. typhimurium* survival in soil.

Treatments analyzed*.	Interaction
All	Time x temperature
All	Time x soil
All	Time x moisture
All	Soil x moisture x temperature
All	Soil x moisture x inoculum carrier
Manure	Soil x moisture
Manure	Soil x moisture x temperature
Saline	Moisture x temperature
Amarillo	Moisture x temperature
Amarillo	Temperature x inoculum carrier
Amarillo	Temperature x moisture x inoculum carrier
Houston Black	Moisture x temperature
Houston Black	Moisture x inoculum carrier

*Only treatments having significant interactions between various factors are listed. The interactions were significant at the 0.05 level by the F test.

Table 4. Population densities of salmonella in two soils incubated at 39°C under two moisture regimes.*

	Amarillo		Houston Black	
	Soil Moisture		Soil Moisture	
Time	-0.5 atm.	<-22 atm.	-0.5 atm.	<-22 atm.
	Number per g soil			
Days				
0	15,000	13,000	15,000	13,000
3	700	300	500	0
7	100	0	0	0
21	40	0	0	0
42	0	0	0	0

*Manure was the inoculum carrier and was mixed into the soils.

Table 5. Population densities of salmonella in two soils incubated at 22°C under two moisture regimes.*

	Amarillo		Houston Black	
	Soil Moisture		Soil Moisture	
Time	-0.5 atm.	<-22 atm.	-0.5 atm.	<-22 atm.
Days	Number per g soil			
0	15,000	12,000	15,000	14,000
3	108,000	1,400	114,000	0
7	11,000	40	6,000	0
21	800	20	1,000'	0
42	45	30	70	0
63	0	0	0	0

*Manure was the inoculum carrier and was mixed into the soils.

of incubation when soil moisture was -0.5 atm. tension. These data strongly illustrate the potential importance of soil and moisture interactions on salmonella survival.

Generally, survival of salmonellae at 5°C was somewhat similar to survival at 22°C (Table 6). However, the various interactions that occurred resulted in many exceptions to this generalization. For the treatment combination of Amarillo and -0.5 atm. tension salmonellae were not detected after 3 days but for the Houston Black, they were detected for 63 days.

The carrier used in inoculating soil with manure generally did not influence the length of time that salmonellae could be isolated from soil. However, exceptions did occur that resulted in significant interactions with other factors. An example of such an interaction is indicated in Table 7. At 22°C salmonellae were not isolated after 7 days when saline was the carrier but when manure was the carrier, salmonellae were isolated for 63 days. The population of salmonellae added by the two carriers was significantly different but cannot account for the large difference in survival for the 22°C treatments. The population of salmonellae increased within 3 days for the 22°C incubation temperature for both inoculum carriers before initiation of a steady decline.

Experiment II

Four soils were used in this experiment to provide additional data on the effect of clay content and soil pH on salmonella survival. Also data were collected to determine if surface application of salmonellae to soil would result in faster death rates than incorporation. Statistical analyses of the data indicated that there were no significant interactions between factors. Therefore only main effects of treatments are presented.

The method of applying salmonellae to soil did not significantly affect the time for 99.9% reduction in population. For surface application 28 days were required for 99.9% reduction in population and for incorporated salmonellae the time requirement was 22 days.

Survival of salmonellae in the Bowie soil was the poorest and 19 days were required for a 99.9% reduction in the population (Table 8). Salmonella survival in the two clay soils was approximately 10 days longer than for the sandy soils. A comparison of survival between the two clay soils and the two sandy soils indicates that soil pH in the range of pH 7.7 to 5.1 did not affect survival. An alkaline and acid soil was included in each textural class (Table 1).

There was no significant difference in time required for 99.9% reduction in the population of salmonellae in soils incubated at 5, 15 or a fluctuating 5-15°C (Table 9). The average time for 99.9% population reduction at these temperatures was 33 days. The population declined

Table 6. Population densities of salmonella in two soils incubated at 5°C under two moisture regimes.*

	Amarillo		Houston Black	
	Soil moisture		Soil moisture	
Time	-0.5 atm.	<-22 atm.	-0.5 atm.	<-22 atm.
Days	Number per g soil			
0	15,000	13,000	15,000	8,300
3	200	500	11,000	540
7	0	30	9,000	400
21	0	0	4,000	0
42	0	0	200	0
63	0	0	15	0
84	0	0	0	0

*Manure was the inoculum carrier and was mixed into the soils.

Table 7. Population densities of salmonellae inoculated into an Amarillo sandy loam, incubated at two temperatures, using physiological saline or manure as the inoculum carriers.

	Saline*		Manure	
	Temperature		Temperature	
Time	5°C	22°C	5°C	22°C
Days	Number per g soil			
0	8,000	8,000	15,000	15,000
3	8,000	13,000	10,000	135,000
7	600	3,000	7,000	18,000
21	200	0	6,000	80
42	50	0	600	25
63	0	0	0	15
84	0	0	0	0

*The population of salmonellae added using the two inoculum carriers was respectively 8,000 and 16,000 for saline and manure. The soil was saturated with water.

Table 8. Time required for 99.9% reduction in the population of _Salmonella_ _typhimurium_ added to four soils.

Soil	Days	
Houston	33[*]	a
Beaumont	28	ab
Amarillo	20	bc
Bowie	19	c

[*] Numbers in columns without letters in common are significantly different (0.05 significance level). Results of main affect from factorial experiment II.

Table 9. Time requried for 99.9% reduction in population of Salmonella typhimurium in soil when exposed to different temperatures.

Temperature	Days	
5°C	29**	a
15°	32	a
5-15	37	a
35	14	b
35-15	12	b

[**] Numbers in columns without letters in common are significantly different (0.05 significance level). Results of main affect from factorial experiment II.

within 14 days when a temperature of 35°C was imposed. A fluctuating temperature of 15-35°C provided results similar to a continuous temperature of 35°C.

An assumption in this experiment was that population density would not influence the rate of decline. An experiment was conducted using the Amarillo sandy loam to determine if this assumption was valid. The initial populations ranged from approximately 9×10^3 to 5×10^6 cells per g of soil. The average time for 99.9% reduction in population was 35 days (range of 30-42 days). The differences were not statistically significant (α=0.5).

To test the difference in survival between isolates an experiment was conducted using three isolates of Salmonellae typhimurium. Inoculum was added in manure to the surface of the Amarillo soil and incubated at 30°C. The organism was the clinical isolate used in Experiment I, the second organism was the feedlot strain used in Experiment II, and the third organism was another feedlot isolate. The results indicated no significant differences in rate of decline between the three isolates. The times required for 99.9% reduction in their populations were respectively 45, 45 and 50 days.

LEACHING OF SALMONELLAE IN SOIL

The population of salmonellae in the liquid added to soil columns ranged between 6×10^5 and 4×10^6 cells per ml. To facilitate comparisons the populations were mathematically normalized to 1×10^6 cells per ml. The density of S. typhimurium in all the column leachates was significantly below 1×10^6 per ml (Figure 2). It is evident from inspection of Figure 2 that increasing the length of the columns decreased the population of salmonellae in the leachate. No S. typhimurium were detected in columns of Houston Black clay that were 2 cm in length or longer. The number of salmonellae in the leachates from 15 cm columns of the Arenosa loamy sand and Angelo sandy clay loam were respectively 1.5×10^3 and 1×10^1 cells per ml. The difference in population reduction of S. typhimurium between the three soils was statistically significant (0.05% level).

The soils showed the same relative ranking for filtering S. enteriditis as S. typhimurium (Figures 2 and 3). Salmonella enteriditis was detected in the leachate from 10 cm columns of Houston Black clay. For the other two soils the populations in the leachates from various columns were quite similiar to populations of S. typhimurium in corresponding leachates. The differences were statistically significant (0.5 level) but probably were close enough to be of little practical significance.

To determine the distribution of salmonellae within columns, 15 cm columns were leached with S. typhimurium and sectioned for enumeration of the populations. The results agreed closely with the data obtained from examining populations in leachates. For the Houston Black clay,

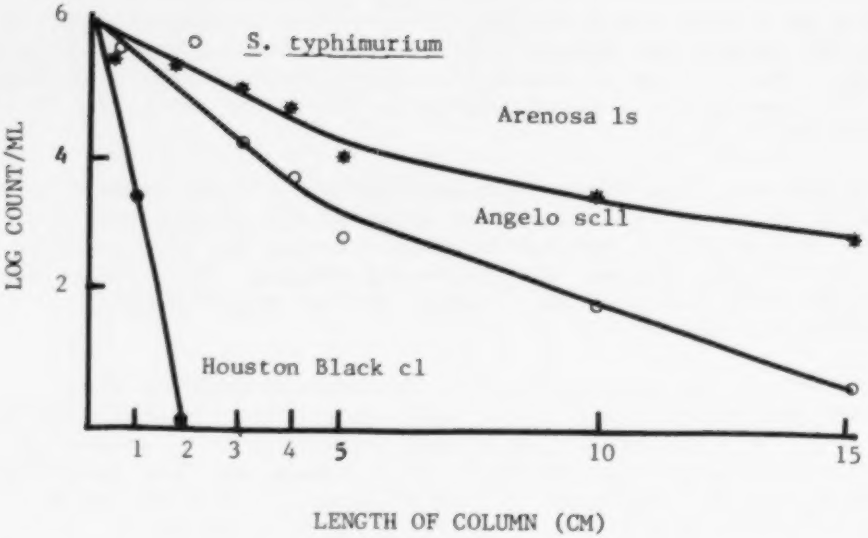

Figure 2. Population of S. typhimurium in leachates collected from soil columns when 5 ml of inoculum was added per cm of column length.

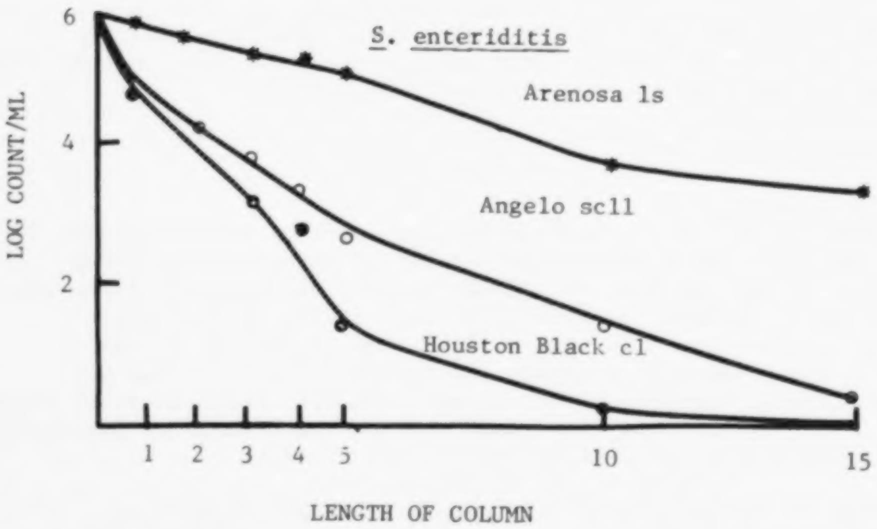

Figure 3. Population of S. enteriditis in leachates collected from soil columns when 5 ml of inoculum was added per cm of column length.

salmonellae were only detected in the 0-3 cm section (Table 10). Salmonellae were distributed throughout the column of Arenosa loamy sand but again the largest percentage (74%) of added bacteria was in the 0-3 cm section. Distribution of salmonellae in the Angelo sandy clay loam also was not even and on a percentage basis 99% of the cells were in the 0-3 cm section.

It is apparent from the results in Figures 2 & 3 and Table 10, that only from 11 to 26% of the salmonellae added to the columns were recovered. Probably this was due to die-off during the time required for leaching, sectioning columns, and processing samples. The conclusions to be reached from this experiment likely are not significantly influenced by the die-off.

The reduction in the population of salmonellae obtained from passing through the soils was likely due to a combination of filtration, adsorption and poor survival. The potential importance of adsorption in reducing the population that may be leached through soil was investigated by separating bacteria adsorbed to 1 um or larger soil particles by differential centrifugation. The density of soil particles is approximately 2g/cc and for bacterial cells it is approximately 1g/cc. Apparently adsorption is potentially very important in the Houston Black clay and the Angelo sandy clay loam because respectively 98% and 82% of the added bacteria were sedimented with the large soil particles (Table 11). For the Arenosa loamy sand adsorption does not appear to be as important because only 11% of the cells were adsorbed. Particles larger than 1 um in diameter were selected for ease of separation from nonadsorbed bacteria and they would probably be large enough to anchor bacteria in place. Smaller particles adsorbed to the bacteria might tend to move with the bacteria. The values for adsorption must be considered as conservative estimates because particles smaller than 1 um would also be important but were not included.

DISCUSSION

The results of these investigations on survival of salmonellae in soil indicate that the organism can survive in nature for extended periods. It is obvious that one cannot predict with any precision the survival time of salmonellae applied to soil in the field. The population will decline with time but the rate of decline is not predictable even when soil moisture and temperature conditions are known. Perhaps an exception would be when the soil temperature is above 39°C and the soil is too dry for plant growth; the salmonella population could be predicted to decrease by 99.9% within 1 week.

Perhaps it is not necessary to require complete die-off of salmonellae in soil within a specified time period. The organism is widely distributed in nature and likely is already present in many environments (Thomason et. al. 1977) at very low levels. The question that needs to be answered is how many salmonellae are permissible in

Table 10. Percentage of applied salmonellae retained in sections of 15 cm soil columns immediately following leaching with a suspension of *S. typhimurium*.[a]

Section	Soil		
	Arenosa	Angelo	Houston Black
0-3 cm	26.0 (±0.1)[b]	11.0 (±0.0)	11.0 (±0.2)
3-5	6.5 (±1.7)	0.12(±0.02)	ND[c]
5-10	1.9 (±0.0)	0.02(±0.01)	ND
10-15	0.8 (±0.1)	0.03(±0.01)	ND

[a] Each column was leached with 75 ml of a suspension containing 1 X 10^6 salmonellae per ml.

[b] The standard deviation for each number is given in parenthesis.

[c] N.D. means no salmonellae detected.

Table 11. Percentage of *S. typhimurium* adsorbed to soil particles larger than approximately 1 μm.

Soil	Percentage
Arenosa	11 (±6.3)[a]
Angelo	82 (±1.9)
Houston Black	98 (±1.4)

[a] Standard deviation in parenthesis.

soil without being a threat to animal or public health? Perhaps that number should not be exceeded in applying wastes to soil. The die-off expected after addition to the soil would reduce the population such that additional wastes could be applied after a waiting period.

The dilution effect that occurs during treatment of municipal sewage during processing results in low populations of salmonellae per unit of waste. The salmonella population in sewage from U.S. municipalities would not likely be as high as for sewage in India where the organism is more prevalent in the population. Joshi et. al. (1973) reported that sewage in a treatment facility in India contained a maximum of 5 salmonellae per ml. The facility was monitored weekly for 6 months. They reported that Gillespie (1944) observed a maximum of 41 salmonellae per ml of raw sewage during a period of high typhoid fever incidence in Java. Dudley et. al. (1980) using enrichment methods isolated salmonellae from sludges obtained from a San Antonio sewage treatment plant and reported that the populations ranged from 2 to <24 per g sludge. Such populations seem relatively low but the ability of salmonellae to reproduce in composted sludges, under moist conditions, (Russ and Yanko, 1981) indicate the population could increase in the sludge before land application.

Addition of 2 acre inches of sewage effluent containing 10 salmonellae per ml to a field would result in approximately 2 salmonellae being added per g of soil in the top 15 cm of soil. From the results of our research it could be conservatively predicted that the population would decline to less than 2 salmonellae per 100 g soil within 6 weeks for any soil condition above freezing. Does this constitute a health hazard? What is the natural level of salmonellae in soil? Such low populations probably cannot even be detected with enrichment methods.

Will applied salmonellae remain in the top 15 cm of soil or be leached to lower depths? Numbers of cells reaching the lower depths would be expected to be reduced by 99% due to adsorption and filtration. The results of our column studies are from an idealized situation. The soils were evenly packed in columns and no cracks or cleavage plains were present as could occur in the field. In the field the bacteria could pass through such channels without filtration occurring. The results of an investigation at the San Angelo sewage farm by Hossner et. al. (1978) and Weaver et. al. (1978) clearly indicates that sewage organisms do leach to lower soil depths. Soil cores collected from the Angelo soil series on the sewage farm were sectioned to determine the distribution of coliforms in the soil profile. The populations in the first 5 cm of the cores, was approximately 1×10^5 coliforms per g of soil and in the 50-100 cm depth the population was approximately 1×10^3 coliforms per g of soil. Coliforms in lower depths of a control soil were not detectable. These results clearly indicate that more leaching occurs in the field than would be predicted from soil column investigations in the laboratory (Figures 2 and 3).

Other evidence that indicates bacteria can be leached through soils in the field was also reported by Weaver et. al. (1978). The population of fecal coliforms added as effluent to soils on the San Angelo sewage farm was approximately 1 x 10^4 per ml. Seepage waters draining the sewage farm contained approximately 2 x 10^1 fecal coliforms per ml. The reduction in population was approximately 99.9% from passing through the soils.

From the evidence reported in this manuscript it appears that it is possible to conservatively predict that for many soils and environmental conditions the population of salmonellae added to soils with sewage sludge or effluent would decline by 99% within 2 months. It also appears that passing the bacteria through 15 cm or more of soil would reduce the population by 99%. The question remains as to how many salmonellae may be added to soil before they constitute a health hazard? It appears to me that addition of sewage effluent to soil in quantities meeting the nutritive and water needs of crop plants would not constitute a public health hazard due to the presence of salmonellae.

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TRANSPORT AND FATE - BACTERIAL PATHOGENS IN SOIL: RICHARD WEAVER

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: Salvato

Do you have any indication from the studies you've made of what the role of soil microorganisms or macroorganisms might have on determining the survival rate of pathogens found in wastewater?

A: Weaver

We haven't looked at biotic factors with regard to salmonella survival. I have observed with other bacteria, rhizobia, that under favorable abiotic conditions in nonsterilized soil the population declines but in sterilized soil under similar conditions the population increased by an order of magnitude. I think that biotic factors would be important in determining salmonella survival. However, harsh environmental conditions like high soil temperature or very low soil moisture would likely be much more harmful.

Q: Lennette

How would you go into the matter of determining how many salmonella you could have in the soil without having a public health hazard? That's an intriguing statement to make, but how are you going to get at the problem of assay and quantitation?

A: Weaver

I don't pretend to know how to do that and in fact it is beyond my area of expertise. Elmer Akin will be speaking on this topic tomorrow. In his abstract he states that approximately

100,000 to 100 million salmonella are required to provide an infective dose for 50 percent of a healthy human population. If sewage sludge and effluent are applied to land at application rates that would take care of crop nutrient and water requirements and if the salmonellae were in the sewage materials at a concentration of 10 per ml, which is very high, the population in the uppermost layer of soil would be approximately two salmonellae per gram of soil. My feeling after reviewing the literature on this topic is that most reviewers conclude there is no real public health hazard involved with applying sewage materials to soils, but because we all know pathogenic organisms are present the real issue is skirted and information on pathogen survival time in soil is sought. In other words, we keep putting the real question off. A related problem is that measuring a salmonellae population of two per g of soil is difficult and can only be achieved by enrichment methods. If we have a 99 percent reduction in population, as indicated from my research, isolation of salmonellae from soil would be extremely difficult. It appears to me that a health hazard, due to salmonellae, from land application of sewage is very unlikely.

Q: Morrison

You commented that you felt that salmonella was ubiquitious in nature. Do you really mean that?

A: Weaver

Well, I guess I have to define what my term is. I'm not all that familiar with trying to isolate Salmonella from a wide range of environments. I only know that it has been reported from rabbits, reptiles, and turtles, and all kinds of things. It was found on the top of Stone Mountain in Atlanta, Georgia. We found it in close to 100% of the feedlots in Texas.

Q: Morrison

I just wanted to get clarification. I think in each of the examples you gave it is animal associated, including the human animal of course; as for tops of mountains, birds do manage to get to the tops. I believe our evidence to date is that Salmonella is animal associated.

A: Weaver

I think that is why it does not survive a lengthy period of time in soil and normally does not reproduce in soil.

Q: Morrison

The other question I have is, how do you feel that working with the geometry of a 25 ml vial compares to the real soil environment? By necessity you worked with vials, other speakers used columns, and I in my own laboratory also, had to work with columns. You always have to face the fact that the soil or sample at the container or column interface creates a completely artificial situation.

A: Weaver

I cannot help but agree with you. Nevertheless, it seems that the principles usually end out the same. There may be large differences between methods but in general the same conclusions will be reached whether a glass vial with 10 grams of soil is used, or if a plastic bag containing 100 grams of soil is used. The results will probably be somewhat different between methods but I think we will still conclude that salmonella survive longer under moist conditions than they do under very dry conditions. Obviously, we could continue these kinds of studies under all kinds of different moisture conditions, temperature conditions, sterilized soils, non-sterilized soils, large vials, small vials and so on, but I wonder how fruitful they would be?

Q: Davidson

I believe you indicated that 80 percent of the liquid added to the column came out, is that correct? That is approximately 80 percent of the added liquid passed through the columns?

A: Weaver

Is that what the manuscript indicates?

Q: Davidson

That's what the manuscript says and I believe you stated that in your talk.

A: Weaver

Yes, I think that is true.

Q: Davidson

How did you achieve that with the Houston clay compared to the sand?

A: Weaver

Well, that is a general statement. It is true that more liquid passed through the sand than the Houston Black clay. When you put 25 ml of liquid onto 5 grams of soil, even for the Houston

Black, the soil is only going to retain 3 or 4 ml. The point I was trying to make was that most of the liquid came out.

Q: Davidson

I believe, based on the moisture characteristic curve, you show about a 50 percent soil-water content in the Houston soil, around 100 centimeters.

A: Weaver

Yes, I indicated the soil contained 50 percent moisture at 100 centimeters water tension. What I am saying is that for 5 grams of soil, I added 25 ml of liquid. There wouldn't be more than 5 mls that could stay in it, so 20 had to come out.

Q: Davidson

The thing that concerns me is that in science you really study processes, and to be able to say what causes something to die or live, or pass through, or not pass through, requires an understanding of very specific processes and you have generalized. You can reach the conclusion that nothing can be described, but good science can't generalize to such a conclusion. I think you have to be careful that you don't generalize to the extent that all processes involving viruses are too complex to be described or their behavior understood.

A: Weaver

Well, I think for the particular issue whether 80 percent passed through or 85 percent is not very important.

TRANSPORT AND FATE: BACTERIAL PATHOGENS IN GROUND WATER

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ABSTRACT

There is accumulating evidence, on a nation-wide basis, that water supplies in many areas of the United States are receiving increased pollution loads, and the source has frequently been traced to contaminated recharge waters. Potential ground water pollutants, including pathogens, are removed from percolating effluents by microbial activity, soil filtration, and adsorption, preventing their entry into ground water. The origins of such effluents are diverse and may include land spreading of wastes, overland flow systems, sewage irrigation, sewage lagoons/holding ponds, and deterioration of older, abandoned disposal sites. In addition, large populations in both rural and urban areas rely on septic tank soil-absorption treatment systems as the principal disposer of domestic waste materials. This paper reviews literature on the mechanisms by which incompletely treated biological wastes may come into contact with various ground water resources. Current information from recent microbiological investigations regarding the transport of effluent-borne bacterial populations through soil in relatively high numbers is the major emphasis of this address. Research needs are identified for problem areas which demand further investigations in order to develop safe guidelines for determining the suitability of biological waste disposal systems in relation to regional soil-water conditions.

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TRANSPORT AND FATE: BACTERIAL PATHOGENS IN GROUND WATER

TEXT

Introduction

Human and animal wastewaters contain bacteria, viruses, protozoa and helminths pathogenic to humans (1). In addition, these infectious agents are widely distributed in most waste effluents and are commonly present in high numbers. Therefore, such untreated wastes embody a potential health hazard and proper wastewater purification and disposal is an important concern (2). For example, septic-tank soil-absorption treatment systems serve as one of the principal disposers of waste effluent to the soil environment (3) and the Office of Water Supply (4), in a U. S. Environmental Protection Agency report to Congress, estimated that three billion cubic meters of domestic wastewater is annually placed in soil subsurface horizons. Regardless of the effluent source, soil percolation of wastes is required for purification before ground water utilized by individual and public water supply wells is replenished. However, many shallow ground water supplies have been polluted by contaminated recharge waters, and several investigators (5, 6, 7, 8, 9, 10) have attributed such a decline in water quality to indiscriminate use of septic tank systems in soils unsuited for adequate domestic waste purification. Septic tank soil-absorption systems are not confined to rural areas of low population density. Across the U.S. there are four counties(Nassau and Suffolk, NY; Dade, FL; and Los Angeles, CA) each with more than 100,000 housing units served by septic tanks, with an additional 23 counties with more than 50,000 household installations (3).

The purification of waste effluents during soil percolation is the optimal condition necessary for maintenance of groundwater quality, yet improper performance of waste disposal systems (or their absence) can result in a malfunctioning of this soil percolation system. The manifestation of soil adsorption dysfunction includes surfacing of untreated effluent, incomplete treatment due to anaerobic conditions, or the subsurface escape of fecal organisms from the treatment zone. In the following sections, most of the examples are taken from reports dealing with septic tank soil-absorption systems because these have been used in most of the recent research on the subsurface movement of fecal organisms into recharge waters.

Purification Efficiency of Soils: A Literature Summary

The soil depths required for removal of enteric bacteria from various effluents have been derived from a somewhat limited body of information. Indeed, research evaluations of the purification efficiency of a much wider variety of soils subjected to many different waste application techniques results in less close agreement on the degree of bacterial retention by soil. The land application of human wastes includes topics which range from the disposal of raw excreta in pit latrines to application of oxidation pond effluents into rapid infiltration basins. In addition, investigations of these various disposal methods serve as the body of knowledge necessary to further define the conditions in which wastewaters are purified by land application, and as such have been the subject of several reviews (11, 12, 13, 14).

Although a wide diversity of disposal methods have been studied (Table 1), and large variations were observed in comparing the results, some pertinent conclusions can be ascertained from this information: 1) coliforms and other microorganisms move only a few dozen cm with the percolating waters in unsaturated soil layers although much greater distances are possible under saturated flow conditions, 2) with all soil water conditions, the degree of bacterial retention by the soil is inversely proportional to the size of the component particles in the unstructured matrix, 3) the physical straining or filtration of organisms by soil particles are the main limitation to travel through soils, and sedimentation of bacterial clusters occurs throughout the zone of saturated flow, 4) adsorption is a factor in the retention of bacteria by soil and becomes more effective in soils with increasing clay content, and 5) death of the microorganism plays an important role during the longer retention periods or unsaturated flow conditions. Thus, both physical removal and inactivation influence the change in bacteriological quality of wastewaters percolating through the soil.

Bacterial Migration from Effluent Absorption-Fields

The most comprehensive study on soil absorption of septic effluent has been conducted by the Small Scale Waste Management Project Group at the University of Wisconsin. In the report of investigations on the purification efficiency of 19 subsurface disposal systems, Bouma et al. (33) concluded that those systems which exhibited proper hydraulic functioning also served to purify septic effluent. Bacterial filtration was determined by dissecting a drainfield and enumerating indicator organisms present in the soil at various distances below the drainfield trench. The large populations of total coliforms, fecal coliforms, and enterococci present in the septic tank effluent were reduced to levels associated with control soil samples within two feet below the percolation trench. Shallow wells were installed strategically in and near the absorption fields, and ground water samples were assayed for indicator organism density and total bacterial counts. Although total bacterial counts were higher in samples taken from wells adjacent to the absorption field and downfield in the direction of ground water flow, in most cases no indicator organisms were detected in these samples. Thus these results indicated that ground water collected adjacent to these treatment systems rendered a minimal health hazard. Reneau et al. (34) investigated the interactions between 1) soils of various suitability for septic tank effluent disposal, and 2) seasonal perched water tables, on the bacterial contamination of a watershed from septic sources. The watershed covered an area of

Table 1. A summation of the results of selected studies on the transport of bacteria through the solumn in relation to land application of domestic wastewaters.

Nature of Pollution	Organisms	Medium	Maximum Distance Traveled	Time of Travel	Reference
Sewage trenches intersecting ground water	*Bacillus coli*	Fine sand	19.8 m (65 ft)	27 wks	15
Sewage trenches intersecting ground water	Coliforms	--	70.7 m (232 ft)	--	16
Sewage in pit latrine intersecting ground water	*Bacillus coli*	Fine and coarse sand	24.4 m (80 ft)	--	17
Sewage in bored latrine intersecting ground water	*Bacillus coli*	Sand and sandy clay	10.7 m (35 ft)	8 wks	18
Sewage in pit latrine intersecting ground water	*Bacillus coli*	Fine and medium sand	3.1 m (10 ft)	--	19
Primary and treated sewage in infiltration basins	Coliforms	Fine sandy loam	0.6-4 m (2-13 ft)	--	20
Diluted primary sewage injected subsurface	Coliforms	Aquifer	30 m (98 ft)	33 hrs	21
Canal water in infiltration basins	*Escherichia coli*	Sand dunes	3.1 m (10 ft)	--	22
Subsurface injection	Enterococci	--	15 m (44 ft)	--	23
Secondary sewage in infiltration basins	Coliforms	Sandy gravels	0.9 m (3 ft)	--	24
Tertiary treated wastewater in Percolation beds	Fecal coliforms and fecal streptococci	Coarse gravels	457.2 m (1500 ft)	15 da	25
Primary sewage injected subsurface	Coliforms	Sand and pea gravel aquifer	30.5 m (100 ft)	35 hr	26

Table 1. (Continued)

Nature of Pollution	Organisms	Medium	Maximum Distance Traveled	Time of Travel	Reference
Secondary sewage injected subsurface	Fecal coliforms	Fine to coarse sand aquifer	30.5 m (100 ft)	--	27
Tertiary treated wastewater in percolation beds	Coliforms	Sand and gravel	830 m (2723 ft)	--	28
Inoculated water and diluted sewage injected subsurface	*Bacillus stearothermophilis*	Crystalline bedrock	28.7 m (94 ft)	24-30 hrs	29
Tertiary treated wastewater in infiltration basins	Coliforms	Fine to medium sand	6.1 m (20 ft)	--	30
Secondary sewage in infiltration basins	Fecal coliforms	Fine loamy sand to gravel	9.1 m (30 ft)	--	31
Primary sewage in infiltration basins	Fecal streptococci	Silty sand and gravel	183 m (600 ft)	--	32

80 ha and contained within its boundaries approximately 30 single-family dwellings and an elementary school. The watershed soils were classified, according to their suitability for septic system effluent disposal, as suitable, marginal, and unsuitable; of the total septic systems placed in this area, 17 percent were in suitable, 41 percent in marginal and 42 percent in unsuitable soils. Nine surface and seven subsurface sampling sites were established throughout the watershed, and samples were collected periodically during the first half of the years 1972 and 1973, when streamflow was continuous and seasonal water tables were present. The bacterial contamination of the watershed from septic systems was evaluated from the total fecal coliform density in the water samples. Bacterial enumeration of a surface water sample which was subjected to minimal contamination yielded fecal coliform most probable numbers (MPN) ranging from 3 to 930/100 ml whereas a surface sample in direct contact with septic effluent contained from 5.3×10^2 to 2.4×10^7 fecal coliform MPN/100 ml. Also, surface samples not in contact with any effluent source had an average fecal coliform MPN of 2.3×10^2/100 ml, while the watershed outflow produced a mean fecal coliform MPN of 5.6×10^3/100 ml sample. Subsurface waters generally displayed a decrease in total and fecal coliform MPN with increased distance from individual septic-tank soil-absorption installations. Contamination of the watershed outflow was particularly noticeable during or following a rainstorm when septic tank effluent was flushed by movement of runoff water into surface flow. Thus, this watershed which contained septic tank systems installed in unsuitable soil yielded runoff water of marginal bacterial quality.

Reneau and Pettry (35) continued this research based on the prior observation that coliform and fecal coliform numbers decreased with increasing subsurface distance from a septic drainfield. Three septic tank drainfields located on different soils were each instrumented with a series of piezometers at selected distances from the drainfield in the direction of ground water flow. The soils, located on the western edge of the coastal plain in Virginia, were considered marginally suitable for the disposal of domestic wastes. In addition these soils, underlain by layers restrictive to water movement, developed perched water tables during periods of seasonally high rainfall. Total and fecal coliforms present in the ground water were enumerated, and the results indicated that although there was a large reduction in bacterial numbers with horizontal distance from the drainfield, high bacterial concentrations were present at all sampling locations. Thus, at 28 m from the drainfield in the Beltsville soil, up to 4.3×10^2 fecal coliform MPN/100 ml were recovered; in the Goldsboro soil, 2.9×10^3 fecal coliform MPN/100 ml were recovered at 13.5 m distance and in the Varian soil series, 1.1×10^5 fecal coliform MPN/100 ml were recovered at 6.1 m from the drainfield. In all cases, the maximum coliform density was observed in the saturated zone directly above the restrictive layers present in these soils, and abrupt decreases in bacterial numbers were observed below these layers. Since few coliforms were observed at the deeper depths in these field sites, and then only on a limited number of sampling dates, the authors concluded that the ground water supply was not being contaminated by vertical movement of fecal bacteria. Yet very large surface areas were required to remove the fecal indicator organisms from effluent as it penetrated horizontally through the soil, and all possible routes of ground water recharge were not investigated. This demonstrated the extended distances that indicator organisms could be transported under saturated flow conditions as compared to unsaturated flow situations.

Artificial drainage has been shown to improve the hydraulic functioning of septic systems located in soils underlain by slowly permeable soil horizons and subjected to seasonally high water tables. Yet health guidelines which specify the minimum distance between the disposal area and intercepting tile lines or drainage ditches were established intuitively with little data support. To address this situation, Reneau (36) examined the influence of artificial drainage on the penetration of total and fecal coliforms into wet, tile drained soils, and drew conclusions to assess the potential health hazards. Sampling wells were installed (at three household systems) to depths of approximately 1.5 m in the area between the disposal field and the drainage tile. The wells were arranged in two rows positioned perpendicular to the septic system drainfield and were composed of four wells per row, placed at increasing distance from the disposal trench, with the final well located on the opposite side of the drainage tile to collect uncontaminated samples. The drainage tiles were located 11.9 m from the disposal trench in site 1, 11.6 m at site 2, and 19.2 m at site 3. Water samples collected during high water table periods displayed a rapid decrease in total and fecal coliforms with increased distance from the disposal area. However, waters in the drainage tiles contained considerably greater total and fecal coliform populations than the control well samples, indicating that horizontal penetration of bacteria in saturated soil could travel long distances. In addition, an adjacent stream receiving the tile flow contained moderately high concentrations of total and fecal coliforms and reflected the influx of septic effluent bacterial populations. Based on the data from this study, the author constructed several hypothetical situations to demonstrate the discharge of fecal organisms into receiving streams from various drainage systems and emphasized the necessity for maintaining an adequate distance between the disposal area and intercepting tile lines.

Viraraghavan (37) positioned a row of 2 m deep ground water sampling wells at distances of 0, 3, 6, 9.2, 12.2 and 15.2 m downgrade from the end of an experimental septic tile line. Virarghavan and Warnock (38) had used this same experimental set-up in their earlier report and noted efficient removal of fecal populations from septic effluents during periods of low water tables. However, the experimentation in the 1978 study was conducted during periods of elevated watertables when only 15 cm of unsaturated soil occurred below the drainfield trench. The results of enumeration of total coliforms, fecal coliforms, and fecal streptococci from ground water samples indicated that these populations also experienced a decline with distance from the septic tile line. Yet fecal coliform numbers at a distance of 15.2 m from the tile line were between 10^2 - 10^4/100 ml sample.

These reports have established that fecal populations present in septic effluent have the potential to penetrate saturated soil, and under certain conditions, be transported various distances through the soils presumably via saturated flow. However, the hydrologic processes responsible for transporting fecal organisms through the soil were not investigated in these static system studies.

Bacterial Transport in Saturated Flow

A preliminary experiment to examine the dynamics of the subsurface migration of fecal organisms was conducted by Hagedorn *et al*. (39). Antibiotic-resistant strains of *Escherichia coli* and *Streptococcus faecalis* were placed (as a biological tracer) into gravel pits positioned in a Veneta soils series located on 2% slope. Sampling wells were located radially around the pit at 0.5 and 1 m distances, and additional wells were located at up to 15 m from the pit in the direction of ground water flow. The characteristics of tracer penetration through the saturated soil profile was determined by enumerating the resistant organisms present in the ground water samples at successive intervals after amendment. The results demonstrated that tracer organisms were intercepted and carried from the gravel pit by saturated flow since successive peaks of bacterial numbers at increasing distance from the pits appeared only after periods of precipitation creating elevated water tables. Although the bacterial density peaks were lessened at increasing sampling distances from the pit, 10^3 - 10^4 cells/ml were detected at even the furthest sampling distance. Also, a second rainfall event produced a secondary set of peaks in the enumeration of tracer vs. time; although the bacterial numbers were not as great. In addition, the organisms survived in the soil water at relative high concentrations for up to 32 days after introduction into the soil.

In a more detailed study, Rahe et al. (40) established simulated drainfield installations on two hillslope soils and monitored downslope bacterial penetration through saturated zones (Figure 1). Antibiotic-resistant *E. coli* were introduced into saturated soil through a horizontal injection line representing a drainfield tile, and ground water was collected at successive periods of time from an array of sampling piezometers located at various depths and distances downslope. In both sites, the flow of tracer bacteria through the soil was characterized by a rapid penetration of the saturated soil with high numbers of organisms contributing to the peak flow (Figures 2 and 3). The bulk of bacterial transport through the soil occurred in specific depth-zones, with the primary transmitting zone located directly above the water-restrictive layer at one site and near the surface layers in the other. These results demonstrated that pathogenic species potentially present in septic effluents could be disseminated through soil in high numbers, over large distances, and after relatively short periods of time (Figure 2). Since bacterial translocation was largely confined to specific zones, Rahe et al. (40) proposed that these highly permeable areas actually represented regions in the solum which contained a large volume of macropores and that partial displacement through these pores accounted for the rapid flow rates.

Using a longitudinal dispersion equation to interpret the dispersal of *E. coli* cells through a porous medium, McCoy and Hagedorn (41) found that the distinctive trends in bacterial numbers recovered over time supported the hypothesis that nearly complete displacement of the amended material had occurred with incoming water. Rapid subsurface movement of the amended bacteria was observed over short time periods for several different soils. The elevated water tables flowed downslope and in the majority of situations entered into a stream or disappeared into subsurface channels while, at a few sites, the water table surfaced downslope and resulted in surface flow into streams and reservoirs.

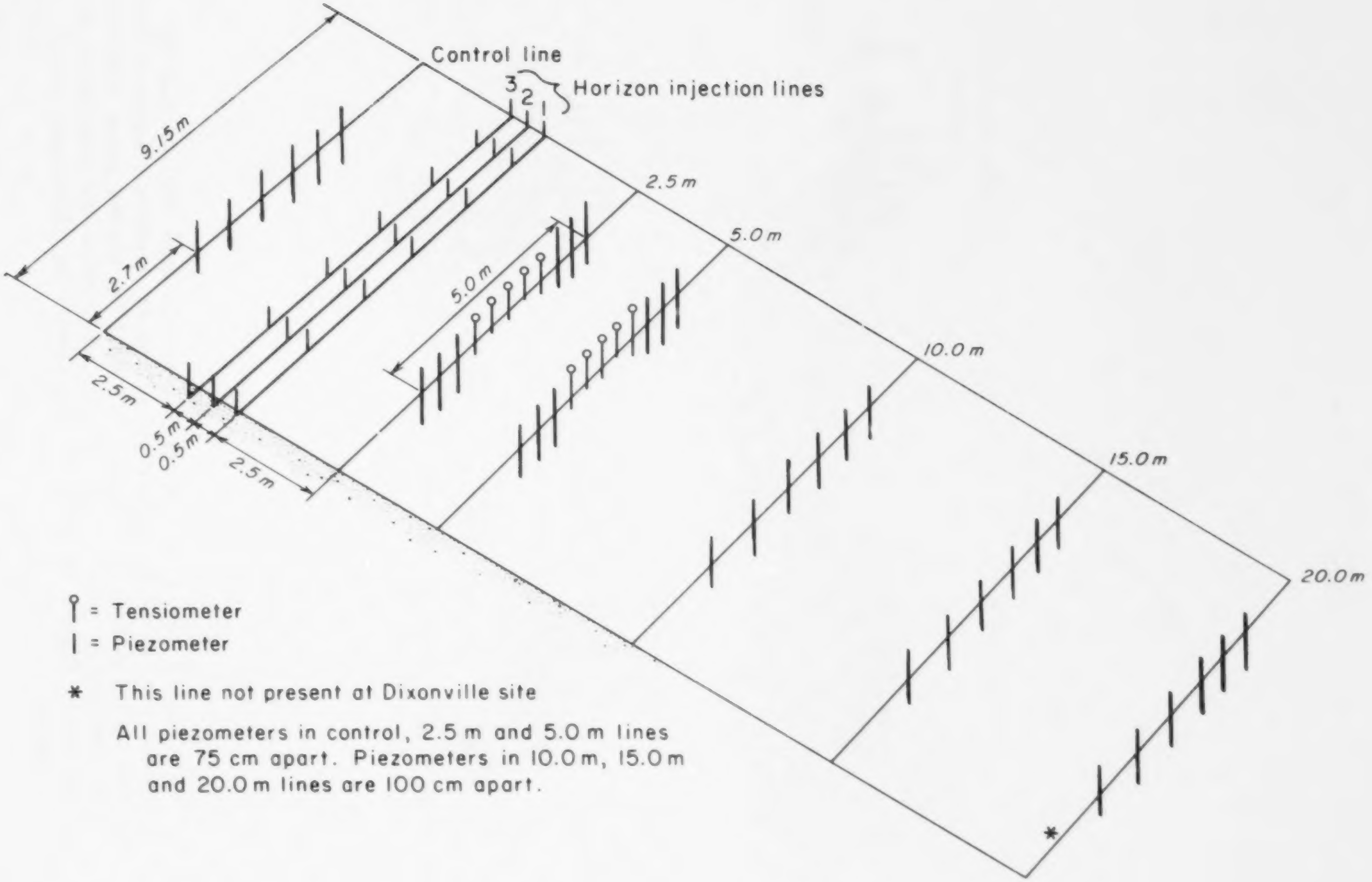
Control line
3
2
1
Horizon injection lines
9.15 m
2.7 m
2.5 m
0.5 m
0.5 m
2.5 m
5.0 m
2.5 m
5.0 m
10.0 m
15.0 m
20.0 m
*
= Tensiometer
= Piezometer
* This line not present at Dixonville site
All piezometers in control, 2.5 m and 5.0 m lines are 75 cm apart. Piezometers in 10.0 m, 15.0 m and 20.0 m lines are 100 cm apart.

Figure 1. Diagram of field site showing locations of piezometers, tensiometers, and sampling lines.

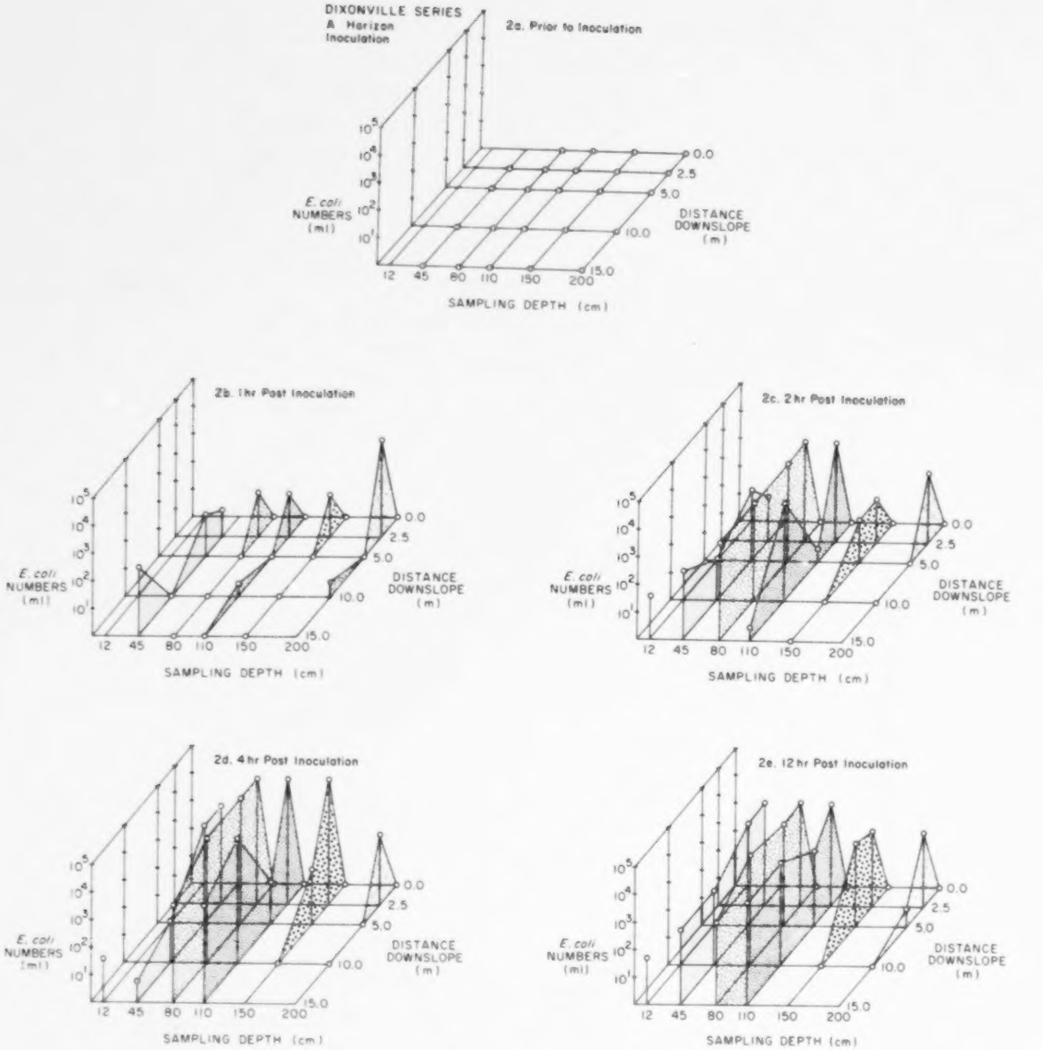

Figure 2. A-horizon inoculation in the Dixonville soil series showing translocation of E. coli populations at various distances through the soil profile with time. The various shaded areas represent the bacterial populations (y-axis, no. cells/ml) recovered at the different depths (x-axis) in the downslope piezometer lines (z-axis).

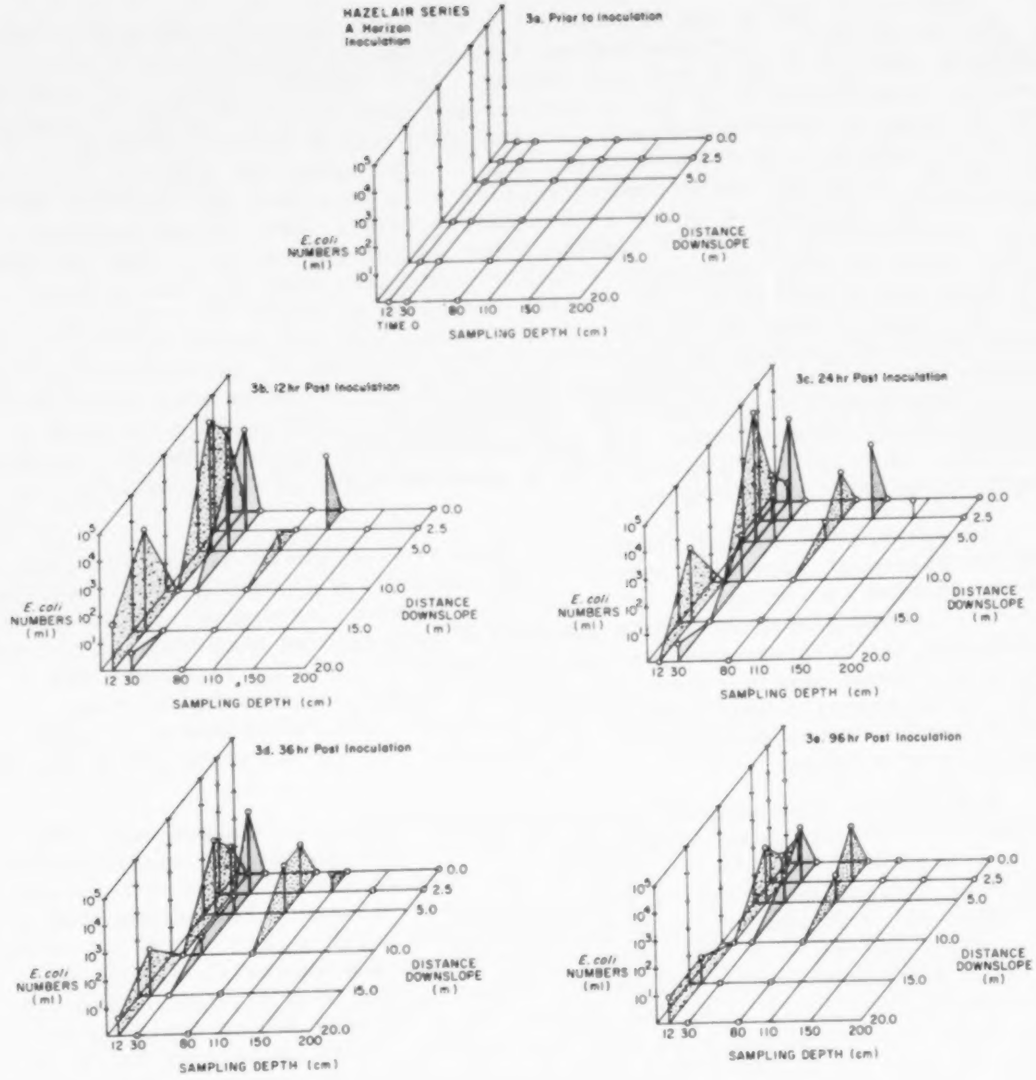

Figure 3. A-horizon inoculation in the Hazelair soil series showing the translocation of E. coli populations at various distances through the soil profile with time. The various shaded areas represent the bacterial populations (y-axis, no. cells/ml) recovered at the different depths (x-axis) in the downslope piezometer lines (z-axis).

The reduction of maximum bacterial counts as a function of distance downslope for the tracer strains injected into the A, B, and C horizons was represented by logarithmic equations (Table 2). These equations were generated from the mean maximum values (N), and the relationships fitted to the transformed values by linear regression analysis.

This study (McCoy and Hagedorn, 41) utilized resistance-labeled strains of *E. coli* to present a phenomenological view of the characteristics of bacterial transport through a saturated soil system. In a similar fashion, effluent-borne bacterial populations from septic systems are also thought to move through the soil in zones of maximum hydraulic conductivity and at rates limited by: (1) the ability of the soil to conduct water, and (2) the hydraulic gradient or slope of the system. Granted, these characteristics will vary from soil to soil and from site to site, however, unifying principles do exist. Even though initially large reductions in bacterial numbers occur as the populations enter the soil system, once the organisms reach a highly conductive zone relatively long distances are necessary for the further reduction of bacterial densities. Effluent-borne pathogens in these circumstances have the opportunity for rapid transport either horizontally to surface receiving waters, or vertically to aquifers through saturated recharge pathways. This evidence indicated that serious contamination of individual water supply systems could occur when a high density of housing units with individual on-site waste disposal systems is located in soils which are generally unsuitable due to the occurrence of seasonally-perched water tables.

In sparsely populated areas, the subsurface escape of fecal organisms from the treatment zone of a septic system drainfield is highly localized and rapidly diluted in the ground water system. However, ground water that meets current coliform standards and is considered safe against outbreads of bacterial disease may not be protected against low levels of virulent pathogens (42). Therefore, proper septic system location and deisgn, especially in areas subjected to elevated water tables and saturated flow conditions which make fecal organism transport possible, is necessary for the adequate protection of potable water sources.

Many states have adopted standards establishing the minimum soil depth to bedrock or highest level reached by a water table in which a septic system can be installed (43). Such standards are based on little or no information concerning the maximum depth of unsaturated soil necessary for proper treatment under all environmental conditions as might exist as any particular location. McCoy and Hagedorn (41) found that the maximum bacterial densities recovered downslope in one soil series varied little from injection into the A horizon (12 cm depth) vs injection into the C horizon (60 cm depth) with a water table approximately 50 cm from the surface. This emphasized the need for a large zone of unsaturated soil around waste treatment systems to prevent the release and rapid transport of large populations of fecal microorganisms.

Summary and Recommendations

Lawes *et al*. (44) reported nearly 100 years ago that a large portion of the water added to moist soils moves immediately through open channels and interacts only slightly with the water in the soil itself. Thomas and Phillips (45) conclusively demonstrated that the flow of water through macropores is important in soil and ground water recharge and in solute movement through soils.

Table 2. Equations describing the reduction in the maximum bacterial densities as observed with injections into the A, B, and C horizons (12, 30, and 60 cm in depth) and subsequent transportation downslope.[†]

Linear transformation	r^2
A horizon injection:	
log (cells/ml) = 8.57 - 1.91 log (distance/cm)	0.97**
B horizon injection:	
log (cells/ml) = 8.79 - 1.66 log (distance/cm)	0.88*
C horizon injection:	
log (cells/ml) = 9.00 - 1.81 log (distance/cm)	0.93**

[†] Determined as the arithmetic mean of the maximum bacterial densities observed from the time of first appearance of the maximum numbers of bacteria and continuing to the final samples.

**Significant at P = 0.005.
*Significant at P = 0.01.

Recent interest in water movement through soil has resulted in reports which show that partial displacement occurs in structured soils when water from irrigation or rainfall flows through macropores (46, 47).

In view of this information, it would appear that the primary research need for waste disposal is the development of reliable soil suitability indices for various disposal systems, and only the Wisconsin Project (33) approaches this end. Soil physical properties such as the formation, distribution and proportion of pore space represented by macropores, the assessment of true saturated hydraulic conductivity rates, and hydrologic relationships of water entering and moving through soil must be quantified. Once basic interactions are identified, these can be used to develop a program wherein a predictive capacity could be included to rapidly evaluate different soils under a variety of moisture conditions.

Septic tank systems rank highest in the total volume of wastewater discharged directly into ground water systems and are the most frequently reported source of ground water contamination (3, 48). One-third of all water-borne disease outbreaks in the United States from 1971 to 1976 were traced to the consumption of water from untreated ground water sources (49) and approximately 60 million consumers rely on the absence of pathogens in untreated ground water (48). With the suburban expansion around metropolitan areas throughout the U. S. into once-rural areas, the trend is set for the exposure of larger numbers of individuals to untreated drinking water. Suburban growth frequently exceeds the expansion of municipal facilities (including water) into newly sub-divided localities, and this makes it essential that on-site waste disposal systems are developed which do not contribute to the deterioration of water quality. This emphasis appears to be necessary because, at present, there is little evidence that other waste disposal systems such as landspreading sewage wastes (1), overland flow, or sewage irrigation systems (50) contribute to ground water contamination to as large a degree. Other methods such as high rate incorporation systems offer a greater potential for ground water pollution and must be designed and maintained to avoid this occurrence.

There are several additional concerns which should mandate some research effort. One important consideration is the development of alternative systems which will function in soils where a conventional septic tank and drainfield are not suitable. Elevated mound systems are one promising possibility (33) and investigations should be expanded on evaluating the longevity, efficiency, and cost of these modified on-site units. Research is needed on techniques to upgrade the performance or efficiency of conventional systems. Examples of such are the use of interceptor tile lines around drainfields to draw off water and pressure distribution to equally disperse effluent over the treatment zone. Deep-plowing to break up the restrictive layers beneath a drainfield is an option which may increase waste retention times and reduce flow through macropores and spaces between the soil peds. Using a fill material as a drainfield bed after removing the original soil is another possibility.

Virtually all of the health-related research in water quality has been centered on fecal indicator bacteria. Efforts are needed to assess the movement potential in soil of other organisms, especially viruses, protozoan cysts, and helminth ova. Many water-borne disease outbreaks are suspected to have a viral etiology when no other causative agent can be identified, and the dynamics of viral translocation through soil may or may not resemble that of fecal bacteria. Critical examination of alternative systems or modifications should include a determination of the retention efficiency of both enteric bacteria and viruses.

Numerous reports in this review have shown the contamination which can occur in surface waters and on a watershed basis through the inefficient operation of conventional systems under unsuitable conditions (34, 40). The National Clean Water Act (Sect. 208 of PL92-500) will require, when standards are enforced, a level of water quality that will not allow a continued deterioration of ground water sources. This is especially true in areas with higher populations (surburban), and even without legislation, alleviation of threats to potable water should be addressed for sanitary and health-related reasons.

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TRANSPORT AND FATE - BACTERIAL PATHOGENS IN GROUND WATER: CHARLES HAGEDORN

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: ________________

Concerning the use of the transport equation which assumes the pollutant is conservative.

A: Hagedorn

We really didn't make that assumption. Perhaps my presentation was not clear. We did make that assumption for these particular experimental systems that we were investigating because the movement was so rapid that first of all there simply was not sufficient time for any appreciable die-off to occur. Secondly, through the rapid movement in these pores which we found when we dissected some of the sites to try and look at adsorption, the indicators simply weren't there. So, yes, that equation was right. It does not take into account adsorption which is why we used it to try to explain the phenomena that we were observing in these particular sites. I would say certainly you would need to consider that if you were going to try and replicate our work.

Q: Davidson

How did you determine that velocity? Did you let the computer select the velocity that provided the best fit to the experimental data when using your equation? If that was the case and you had linear adsorption, then the velocity you report may not be the real velocity of the water but rather that of the retarded solute.

A: Hagedorn

Jim, we tried. There was a study that preceded ours which used chloride and nitrates. We tried to use both of these, our organisms and a couple of indicator dyes to determine the velocity in water. For all five of these indicators we obtained a different velocity.

TRANSPORT AND FATE OF VIRUSES IN SOILS

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ABSTRACT

The transport and fate of enteric viruses in soils is an important public and environmental health consideration in the treatment and disposal of human and animal wastes. The nature and degree of virus retention by soils and inactivation in soils will influence the extent of virus contamination of both the soil and the associated ground water. The purpose of this paper is to highlight and provide an update of available information in this area and to identify critical areas for further research. Literature is reviewed under two broad categories: first, the factors influencing virus survival in soils (temperature, pH, moisture content, soil type, virus type, virus aggregation, salt species and concentration, microbial and related chemical activity and virus association with soil and other particulate matter) and second, the factors influencing virus transport and retention in soils (pH, organic matter, soil composition, virus type, salt species and concentrations, virus association with particulate matter and virus aggregation, and hydraulic conditions and moisture content).

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TRANSPORT AND FATE OF VIRUSES IN SOILS

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INTRODUCTION

The transport and fate of enteric viruses in soils is an important public and environmental health consideration in the treatment and disposal of human and animal wastes. There are over 100 different human enteric viruses that can be present in feces and exudates from infected people (23, 46). Both wild and domestic animals also can become infected with and hence excrete additional enteric viruses, some of which may cause illness and infection in humans. Virus concentrations in feces may be as high as 10^6 infectious units per gram for enteroviruses (55, 69) and 10^9 virus particles per gram for rotaviruses (34), although not all of the latter may be infectious. Enteric virus concentrations in raw sewage in the United States may sometimes be as high as 10^5 infectious units per liter (17), and conventional sewage treatment systems are not likely to reduce raw sewage virus levels by more than 99% (43).

Potential human and animal health hazards from enteric viruses are associated with a variety of purposeful and inadvertant situations where virus-laden wastes may gain access to soils and groundwaters. These include land application systems for wastewaters and sludges, such as groundwater recharge operations, landscape and crop irrigation systems and on-site septic waste disposal units. Additional sources of enteric viruses in soils and groundwaters of an inadvertant nature are: infiltration of fecally contaminated surface waters and surface runoff from precipitation; leachates from pervious wastewater ponds and basins, from sewage sludge spreading, drying and composting operations, and from solid waste landfills and dumps; and leakage from sewer lines. Evidence for virus contamination of soils and groundwaters from such sources comes from both the occurence of waterborne virus disease outbreaks (12) and the direct isolation of viruses from soils and groundwaters (33).

The nature and degree of virus retention by and inactivation in soils will influence the extent of virus contamination of both the soil and the associated groundwater. Although information on virus transport and fate in soils is still inadequate and incomplete, many of the controlling and influencing factors have been identified and evaluated, especially in laboratory and pilot scale studies. This subject has previously been reviewed in considerable detail (3, 16, 22) but new findings continue to be reported in the literature. Therefore, the purpose of this paper is to highlight and provide an update of available information and identify critical areas for further research.

VIRUS INACTIVATION IN AND RETENTION BY SOILS

In considering virus transport and fate in soils, it is useful to distinguish between those factors and conditions in the soil environment that will play a role in virus inactivation or protection from inactivation and those that influence virus movement or retention. Some conditions or factors may influence both the inactivation and retention of viruses while others may predominantly influence only one of these two general phenomena. It is especially important to recognize that under some conditions, viruses may be extensively retained in soils but not be appreciably inactivated. This possibility is of concern because such persistence may lead to extensive accumulation of infectious viruses in the soil. If these accumulated viruses were subsequently released from the soil as a result of changing conditions, considerable groundwater contamination could occur. In fact, such release and migration of accumulated viruses in soils as a consequence of changing soil conditions has been demonstrated in laboratory and pilot scale studies with soil columns (15, 37, 40, 62) and under field conditions (68). The factors and conditions influencing virus survival in and retention by soils will be separately discussed.

FACTORS INFLUENCING VIRUS SURVIVAL IN SOILS

The important factors that have been identified as influencing virus survival in soils are summarized in Table 1.

TABLE 1. FACTORS INFLUENCING VIRUS SURVIVAL IN SOILS

Temperature

Microbial Activity and Related Chemical Activity

Moisture Content

pH

Salt Species and Concentrations

Virus Association with Soil and Other Particulate Matter

Virus Aggregation

Soil Type (textural, chemical and mineralogical properties)

Virus Type

For many of these factors the precise mechanisms by which they cause virus inactivation or protection have not been fully determined. Furthermore, it must be recognized that natural soils are dynamic ecosystems and that the interactions between many of these factors add to the complexity of elucidating their precise roles in virus inactivation or protection. Much of what is known about the effects of these factors on virus survival in the environment is based upon studies in water, wastewater and sludge. Some of the mechanisms by which these factors affect virus survival may be similar in these different media, but the unique characteristics of soil systems may cause them to have qualitatively and quantitatively different effects in soils.

Temperature

Temperature has a considerable influence on virus inactivation rates in soils. As shown by the data summarized in Table 2, the survival times of viruses in soils are shorter at higher temperatures.

TABLE 2. EFFECT OF TEMPERATURE ON VIRUS INACTIVATION RATES IN SOIL MATERIALS

Virus Type	Soil Type	Water or Sewage Type	Approx. No. Days for 99% Inactivation at: 1-8°C	20-25°C	Reference
Poliovirus type 1	Sand	Tap water	>>175[a]	42[a]	42
	Sand	Treated effl.	>>179	51	
Poliovirus type 1	Sand	Septic tank effluent	416	27	26
Poliovirus type 1	Loamy fine sand	Treated effluent	>>84	20	15
Poliovirus type 1	Sand	Dist. water	>>75[a]	50[a], 56[a]	30
	Sand	Dist. water	>>75	14[b], 50[c]	
Poliovirus type 1	?	?	>>134	77	56

[a] Sterile soil columns

[b] Non-sterile, aerobic soil columns

[c] Non-sterile, anaerobic soil columns

Thus, viruses are likely to persist longer in soils during the colder months of the year. It should be noted from Table 2 that the data of Lefler and Kott (42) and some of the data of Hurst et al. (30) were obtained with sterile soil samples, so in these cases the observed temperature effects cannot be attributed to differences in soil microbial activity at the different temperatures.

Other investigators have also reported greater virus inactivation rates at higher temperatures. Yeager and O'Brien (70) found that coxsackievirus B1 inactivation rates in sandy and sandy loam soils suspended in river water, groundwater and septic wastewater increased as temperatures were increased from 4 to 37°C. In pilot scale outdoor studies on poliovirus persistence on vegetables and in soils irrigated with sewage effluent in Cincinnati, Ohio, Larkin et al. (41) and Tierney et al. (65) found that 99% inactivation in soils took about two months during the winter months and only 2-3 days in the warm summer months of June and July. In a field study by Hurst et al. (31) on virus survival and movement in a rapid infiltration system for wastewater, the rate of inactivation of indigenous viruses was greater in the fall than in the winter, possibly due in part to the effects of higher temperatures in the former season.

The mechanisms of virus inactivation due to thermal effects have not been carefully studied in soils, but the findings from studies in aqueous solutions may be relevant. Based upon studies with poliovirus, Dimmock (13) proposed a general model for thermal inactivation in which there are different mechanisms at "low" (<44°C) and "high" (>44°C) temperatures. At low temperatures the loss of poliovirus infectivity in buffered water corresponds to the inactivation rate of viral RNA. At "high" temperatures, the rate of virus inactivation is greater than the rate of RNA inactivation and is associated with structural changes in virus capsids. These findings have been confirmed by other workers (7). Because soil temperatures rarely if ever reach 44°C, it is likely that thermal inactivation proceeds by the "low" temperature mechanism. However, there may be additional thermal mechanisms of virus inactivation in soils.

Soil Moisture

Soil moisture also influences virus survival in soils, although there is somewhat conflicting information in the literature on the effects of this factor. Lefler and Kott (42) observed approximately the same rates of poliovirus inactivation in dried and saturated sand, but the amount of soil moisture in the dried sand was not specified. Other workers have reported more rapid virus inactivation in dried than in wet soils. Bagdasaryan (1) reported that enteroviruses survived 3 to 6 times longer in soils with 10% moisture content than in air-dried soils. Sagik et al. (56) found that poliovirus type 1 was inactivated considerably more rapidly in drying soil as the moisture content decreased from 13 to 0.6%, than in the same soil type maintained at 15 or 25% moisture content. Inactivation of 99% of the initial viruses occurred within 1 week in drying soil but took 7-8 and 10-11 weeks in soils with 25 and 15% moisture content, respectively.

Yeager and O'Brien (70) compared the degree of poliovirus inactivation in eight different soils saturated with river water, groundwater or septic wastewater and in the same soils that were allowed to dry out during the course of the experiment. Upon drying, none of the initial viruses were detectable in any of the dried soils (>99.999% inactivation), but considerable quantities were still present in the same types of saturated soils. In experiments on the rate of poliovirus inactivation at different soil moisture levels, there was a sharp increase in the inactivation rate at 1.2% soil moisture compared to that at 2.9%.

Hurst et al. (30) also observed differences in poliovirus inactivation rates at different soil moisture levels, with the greatest inactivation rate at a moisture level of 15%. Inactivation proceeded more slowly at both higher and lower moisture levels, but the slowest inactivation rates were at the two lowest moisture levels, 5 and 10%.

In a field study on virus survival in a rapid infiltration system for wastewater, Hurst et al. (31) found that virus inactivation rates were greater in more rapidly drying soils. Allowing soils in rapid infiltration systems to periodically dry and become aerated between wastewater applications enhances virus inactivation. The effects of both drying and aerobic microbial activity may contribute to virus inactivation under these conditions. In studies on the mechanisms of virus inactivation in soils, Yeager and O'Brien (71) found that the loss of poliovirus infectivity in moist and dried soils resulted from irreversible damage to the virus particles, including: (a) dissociation of viral genomes and capsids, and (b) degradation of viral RNA. In both moist and dried, non-sterile soils, viral RNA was released from capsids and found in a degraded form. In dried, sterile soils, viral RNA was released but remained largely as intact molecules. Viral capsid components were not readily recoverable from drying soils due to irreversible binding, but they could be recovered as empty capsids from moist soils. Further experiments with dried viruses showed that their capsids became isoelectrically altered. The results of these studies suggest that poliovirus and perhaps other viruses are inactivated by different mechanisms in moist and drying soils.

Soil Microbial Activity and Related Chemical Activity

Soil microbial activity and perhaps related chemical activity due to microbial enzymes and other chemicals appears to decrease virus survival in soils. In an early report, Bagdasaryan (1) noted greater enterovirus inactivation in non-sterile than in sterile sandy and loamy soils, incubated at 3-10 and 18-23°C. In more recent studies by Sobsey et al. (62) on rates of poliovirus and reovirus inactivation in 8 different soil suspensions in settled sewage at 20°C, the time required for 99% inactivation was almost always shorter in non-sterile than in sterile suspensions (Table 3). Hurst et al. (30) observed increased inactivation of poliovirus and echovirus in non-sterile sandy soil wetted with distilled water and incubated under aerobic conditions at 23 and 37°C, compared to sterile control samples. However, inactivation rates in sterile and non-sterile samples were similar at 1°C under aerobic conditions and at 1, 23 and 37°C under anaerobic conditions. Thus, appreciable virus inactivation due to microbial activity in soils appears to occur only under aerobic conditions and at moderate to high temperatures.

Although the mechanisms of microbially-mediated antiviral activity in soils have not been fully elucidated, Yeager and O'Brien (71) have reported differences in poliovirus structural changes during inactivation in sterile and non-sterile soils depending upon soil moisture level. In both sterile and non-sterile soils under moist conditions, viral RNA was probably damaged before release from capsids. In sterile, dried soils released RNA genomes remained largely intact, but in non-sterile dried soils the released RNA was degraded. The role of microbially produced nucleases in these findings is uncertain.

TABLE 3. VIRUS SURVIVAL IN NONSTERILE AND STERILE SUSPENSIONS OF SOIL MATERIAL IN SETTLED SEWAGE[a]

Sample	Time for 99% Virus Inactivation (days)			
	Poliovirus		Reovirus	
	Nonsterile	Sterile	Nonsterile	Sterile
Control	34	86	9	38
Fripp	25	69	20	113
Lakeland	47	154	23	61
Ponzer	9	18	17	146
Bentonite	37	83	23	8
Kaolinite	49	95	30	257
Cecil (B)	80	167	110	233
Davidson (B)	46	73	35	111
Norfolk (B)	47	109	51	139
Mean	42	95	35	123

[a] Samples contained 5% (wt/vol) soil material, except bentonite and kaolinite, which contained 1%. Controls contained only settled sewage with virus. Temperature = 20 ± 1^{o}C; pH 6.5, except Ponzer = 5.5.

Studies on the role of microbial activity on virus inactivation in natural waters and biological sewage treatment processes may also be pertinent to microbially mediated virus inactivation in soils. Herrmann et al. (28) found that enterovirus inactivation proceeded more rapidly in non-sterile lake water in dialysis bags *in situ* than in sterile-filtered lake water held under similar conditions in the laboratory. Virion degradation and probable microbial utilization of virion structural proteins as substrate was also observed. Cliver and Herrmann (10) found that laboratory cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis* inactivated certain enteroviruses, possibly through the activity of microbial enzymes and other antiviral chemicals produced in the cultures. Virion structural proteins were degraded and used as substrate by the bacteria. Furthermore, purified microbial enzymes from *Streptomyces griscus* and subtilisin and neutral pronase from *B. subtilis* inactivated certain enteroviruses.

In studies on poliovirus inactivation in the activated sludge process, Malina et al. (44) reported that the viruses rapidly adsorb to the activated sludge solids but their loss of infectivity proceeds at a slower rate. When adsorbed virions become inactivated, their RNA is released back into the activated sludge supernatant.

Information in the literature indicates that aerobic microbial activity in soils plays a role in virus inactivation, but considerably more work is needed to determine the nature and extent of this activity and the contributing roles of other soil conditions that influence microbial activity.

Effects of Ionic Salts and pH

The direct effects of ionic salts and pH on virus survival in soils have been less extensively investigated than their effects on virus retention by soils. Murphy et al. (51) found that mouse encephalomyelitis virus was inactivated more rapidly in soil adjusted to pH 3.7 and 8.5 than in neutral soil. More recently, Hurst et al. (30) determined that virus inactivation in soils correlated with soil levels of resin-extractable phosphorous, exchangeable aluminum and soil pH. Because these same factors also influence virus adsorption to soils, the observed differences in survival rates may be related to changes in the extent of virus adsorption to the soil material and therefore, changes in the extent of virus protection from inactivation in the adsorbed state. As indicated in the next section of this paper, viruses adsorbed to soils may in some cases survive longer than freely suspended viruses.

Direct effects of different pH and salt levels on virus survival in aqueous solutions have been reported by Salo and Cliver (57), and similar phenomena may occur in the soil environment. They observed that poliovirus type 1 was inactivated more rapidly at the pH extremes of 3 and 9 than at the intermediate levels of 5 and 7. Virus inactivation rates also increased with increasing concentration of NaCl, and the effect was most pronounced at pH 3. Under conditions of equal ionic strength, different inorganic salts produced different inactivation rates, thus indicating specific ion effects. At all pH levels tested, the RNA of inactivated virions became ribonuclease-sensitive, and at pH 5 and 7, the RNA was hydrolyzed in the absence of ribonuclease. These findings suggest that some alteration of the virus capsid occurs when virions are inactivated. The proteolytic enzyme chymotrypsin degraded the capsids of poliovirus inactivated at pH 3, but not at the other pH levels tested.

All viruses do not respond to pH and salts in the same way, because coxsackievirus A9 was inactivated more rapidly at pH 5 than at the extremes of 3 and 9. Further evidence for different enterovirus responses to salt effects comes from studies by Cords et al. (11) who found that a number of type A coxsackieviruses were inactivated more rapidly in low ionic strength solutions than in high ionic strength solutions. Inactivation was accompanied by the loss of capsid protein VP-4. However, this loss did not occur with inactivated poliovirus type 1 or coxsackievirus B.

Another example of the effects of pH on virus survival is provided by studies with adenoviruses. Studies in this author's laboratory by Jacobs (32) showed that the simian adenovirus SV-11 was more rapidly inactivated at pH 5.0 and 6.0 than at pH 7.0 and 4.0. Prage et al. (53) found that the inactivation of type 2 adenovirus exposed to water buffered at pH 6.0-6.6 was accompanied by an increase in DNAase sensitivity and the loss of structural proteins from the vertices of the virion capsids.

Although the results of these studies indicate that virus inactivation can be caused by pH and salt effects, the extent to which these factors contribute to virus inactivation in soils is uncertain and deserves investigation.

Soil Type, Virus Association with Soils and Virus Aggregation

The association of viruses with soil particles may either enhance or reduce their survival, depending on the chemical properties of the soil particle. A number of workers have observed that virus adsorption to soil particles prolongs their survival. Gerba and Schaiberger (21) found that bacteriophage MS-2 survived longer in seawater when associated with kaolinite clay than in clay-free water. Although the mechanisms of protection were not established, it was speculated that kaolinite adsorption of antiviral chemicals, stabilization of virion structure, or changes in the extent of virus aggregation could be responsible. Bitton and Mitchell (4) observed that bacteriophage T7 survival was prolonged when adsorbed to montmorillonite clay or *Escherichia coli* cells.

Adsorption to soil particles does not always result in enhanced survival, and, in fact, may in some cases cause enhanced inactivation. Studies in this author's laboratory showed that poliovirus and reovirus in suspensions of 8 different soil materials did not always survive longer than in soil-free controls (Table 3)(62). Murray and Laband (52) found that poliovirus adsorption to oxide particles of manganese, aluminum and copper resulted in significant and rapid inactivation, while adsorption to silica and iron oxide did not result in significant inactivation.

Recent batch studies by Moore et al.(48, 49) indicated that reovirus adsorption to several soil materials, including Ottawa sand, dolomite, montmorillonite and organic muck resulted in considerable inactivation. Using radiolabeled viruses, it was found that viral radioactivity in the solution phase increased or stayed the same after 1 or 24 hours of adsorption, thus suggesting that adsorbed viruses may have been subsequently released from soils but were no longer infectious. Virions in solution had a normal buoyant density as determined by centrifugation and therefore had not undergone gross physical changes.

Virus aggregation tends to enhance the survival of viruses exposed to various antiviral agents, and this phenomenon has been extensively studied in chemically defined solutions containing purified virions and known quantities of specific disinfectants (61, 72). Although there are no definitive reports concerning the effects of aggregation on enteric virus survival in soils, the results of studies in water suggest that virus aggregates would survive better in soils than would dispersed viruses.

Virus Type

Because different viruses vary in their susceptibility to inactivation by a variety of physical, chemical and biological agents, variations in their ability to survive in soils is not unexpected. The results of an early study by Bagdasaryan (1) indicated some differences in the survival times of five enteroviruses in sandy and loamy soils. Lefler and Kott (42) noted that bacteriophage f_2 survived longer than poliovirus in either dry or saturated sand. Sobsey et al (62) found that the rates of poliovirus and reovirus inactivation in suspensions of 8 soils in settled sewage were often quite different, as shown by the results summarized in Table 3. Hurst et al. (30) reported different inactivation rates among enteric viruses in a sandy soil wetted with sewage. Coxsackievirus A9 was inactivated most rapidly (>90% in one day) and coxsackievirus B3 was inactivated least rapidly (90% in about 15 days). In contrast, however, Hurst et al. (31) noted that the rates of poliovirus type 1 and echovirus type 1 inactivation were generally similar under field conditions in a rapid infiltration system. Additional studies are needed to quantitatively determine the range of differences in enteric virus survival rates for different soils and soil conditions.

Soil Organic Matter

The effects of organic matter on enteric virus survival in soils have not been established, but recent findings suggest that fulvic and humic acids may mask virus infectivity by a reversible process. Bixby and O'Brien (6) found that fulvic acid complexation of bacteriophage MS2 caused considerable loss of infectivity and prevented adsorption to soil. The infectivity of the complexed phage could be restored by treating with 3% beef extract solution at pH 9. Recent results from the author's laboratory (A. Hickey and M.D. Sobsey, unpublished results) indicate that humic acid solutions in water cause loss of poliovirus infectivity (Table 4). Although the extent to which the loss of infectivity is chemically reversible has not yet been determined, at least some of the lost infectivity is restored when the samples are passed through microporous filters, presumably by disruption of the humic acid-virus complexes. Further studies on the effects of soil organic matter on virus survival are needed.

TABLE 4. EFFECT OF HUMIC ACID ON POLIOVIRUS INFECTIVITY

Humic Acid conc. (mg/ℓ)	% Control Virus Detected
0 (control)	(100)
3	91
10	84
30	36

Humic acid solutions in activated carbon-filtered tap water, pH=7.5. Initial virus concentration ≈ 2 x 10^4 PFU/ml.

FACTORS INFLUENCING VIRUS TRANSPORT AND RETENTION IN SOILS

The ability of soils to retain viruses and the factors influencing virus transport and retention have been studied in some detail and this literature has been previously reviewed (3, 16, 22). These factors are listed in Table 5, and they will be discussed here with an emphasis on recent findings and critical needs for further research.

TABLE 5. FACTORS INFLUENCING VIRUS TRANSPORT AND RETENTION IN SOILS

Virus Association with Particulate Matter and Virus Aggregation

Soil Composition

Virus Type

pH

Salt Species and Concentrations

Organic Matter

Hydraulic Conditions and Moisture Content

Virus Association with Particulate Matter and Virus Aggregation

Like other small particles, viruses can potentially be removed and retained by soils by three mechanisms: (a) straining or entrapment either at the soil surface or at contact junctions between subsurface soil particles; (b) sedimentation at the soil surface or on the downstream side of soil particles as a result of velocity decreases that cause suspended matter to settle out; and (c) adsorption by physical-chemical interactions between virion and soil particle surfaces (47). For free virions, that is, virions not associated with larger particles or with each other as aggregates, the first two mechanisms are likely to be of minor importance. Free virions are probably removed in soils primarily by adsorption. However, many viruses in sludges, water and wastewater are associated with either particulate matter or each other (2). If such virus-containing particles are much larger than single virions, they could also be removed by straining and sedimentation. In field studies on virus movement in a rapid infiltration system for wastewater, Hurst et al. (31) noted that viruses accumulated in the wastewater sludge layer that developed on the soil surface as well as in the upper soil levels, possibly because the viruses were already solids-associated and removed by filtration or because they became adsorbed to the sludge solids and soil particles. The relative importance of the different mechanisms of virus removal by soils is largely unknown and has not been adequately investigated in either laboratory or field studies.

Soil Composition

Soils very considerably in their textural, chemical and mineralogical properties and hundreds of soil types have been classified and mapped in this country (66). Furthermore, both vertical and horizontal variability is a normal characteristic of many soils.

A number of studies have shown that the extent of virus transport through soils varied with the type of soil material. In an early study on poliovirus removals from oxidation pond effluent by columns of three soil materials, Laak and McLean (35) found that removals were greatest with sandy loam, least with sand and intermediate with "garden soil". Hori et al. (29) found that poliovirus removals from distilled water in 6-inch columns of three Hawaiian soils, including two, low-humic latosols (Lahaina and Wahiawa) and a volcanic cinder (Tantalus), averaged >99, >99, and 22%, respectively. With the two, low-humic latosols, there was a trend of decreased retention over the 5-day test period. Goyal and Gerba (25) noted considerable differences in the abilities of 9 different soils to adsorb a number of enteric viruses. Statistical analysis indicated that pH was the most important soil characteristic influencing virus retention, with soils having a pH <5 giving consistently high retention. Exchangeable aluminum was another factor that correlated with the adsorption efficiency of many of the viruses. Burge and Enkiri (8) found variations among five different soils in adsorbing bacteriophage ØX174. With four of the five soils, adsorption rates correlated with cation exchange capacity, specific surface area and organic matter content.

In studies by Sobsey et al. (62) the retention of poliovirus by 4-inch long columns of four soil materials intermittantly receiving settled sewage varied with soil type. Sandy (Fripp and Lakeland) and organic (Ponzer) soils gave virus removals of only 98-99.7%, but a sandy clay loam (Norfolk) gave removals of $\geq$99.996% (Table 6).

TABLE 6.

Soil	Mean % Reduction of	
	Poliovirus	Fecal Coliforms
Fripp	98	99.996
Lakeland	99.7	99.995
Ponzer	98.5	99.999
Norfolk (B)	>99.996	>99.9998

Columns were 2.6 cm dia. and 10 cm long. Dosed with settled sewage containing $\approx 3 \times 10^5$ PFU/ml at a mean rate of about 1 cm per day.

In contrast, fecal coliform bacteria removals were much greater in all four soils, averaging about 99.996% for the two sands, 99.999% for the organic soil and > 99.9998% for the sandy clay loam. These findings suggest that fecal coliform bacteria may not be suitable indicators of enteric virus retention in soils.

Moore et al. (50) recently reported that poliovirus type 2 adsorption to 34 different soil material suspended in a synthetic fresh water was negatively correlated with soil organic matter content and with available negative surface charge as measured by adsorption capacity for a cationic polyelectrolyte. Soil pH, surface area and elemental composition were not significantly correlated with virus adsorption. Furthermore, additional studies by this same group indicated that the two poorest adsorbents for both poliovirus and reovirus among 34 different soil materials were a muck soil and a silt loam, both of which had high organic matter content (18, 48, 49, 63).

The results of these studies indicate that soil type greatly influences the extent of virus transport or retention. It may be possible to distinguish soils into general classes with respect to virus retention, based upon their textural, mineralogical and chemical properties. However, further studies with a wide range of soil types and viruses are needed to determine if such classifications are possible and to identify the soil characteristics that most influence virus retention.

Virus Type and Strain

Recent studies have shown that different types and strains of viruses are not equally retained by soils. These virus-specific differences in adsorption to soils are probably related to physico-chemical differences in virus capsid surfaces. Although all enteric viruses possess outer capsids comprised of polypeptide subunits and generally behave as charged, amphoteric, colloidal particles, the surfaces of the virions differ in the details of their configuration, charge density and distribution, and other features. In fact, even the same virus can display different surface properties that will influence their physico-chemical behavior as a result of conformational changes brought about by pH effects and interactions with soluble chemicals and particulate surfaces (19, 45, 71).

Goyal and Gerba (25) found that different enteric virus types and strains varied in their ability to adsorb to soils. For example, adsorption efficiencies of six different strains of echovirus type 1 in suspensions of sandy soil in deionized water ranged from 0 to 99.7%. Type and strain dependence of enterovirus adsorption to a sandy loam soil suspended in distilled water was also reported in another study from the same laboratory (24). Adsorption efficiencies of ten different virus types and strains ranged from 0% for echovirus type 1, strain V239, and coxsackievirus B4, strain V216, to 99.9% for echovirus type 7, Wallace strain, and poliovirus type 1, strain LSc. Landry et al. (39) reported type and strain differences in enterovirus adsorption to sandy soil cores. Vaccine strain poliovirus type 1 (LSc), a widely employed enterovirus model in soil and other environmental studies, was efficiently adsorbed but not readily eluted with either distilled water or sewage effluent. Some of the other enteroviruses tested, including field strains, were less efficiently adsorbed and more easily eluted. It was concluded that vaccine strain poliovirus type 1 may be an inappropriate model for studying the nature and extent of virus transport in soils.

In contrast to the findings from batch laboratory studies by the same group, Hurst et al. (31) found that under field conditions at a rapid infiltration site, echovirus type 1, Farouk strain, did not migrate as far down in the soil as poliovirus type 1, strain LSc. They suggested that the adsorptive behavior of viruses in laboratory batch studies may not be totally reflective of their behavior under field conditions, possibly because of virus adsorption to soil particles prior to infiltration.

Because these studies indicate that different enteric viruses are not transported or retained in soils to the same extent, additional research with a wide variety of viruses is needed to establish the range of differences in virus transport through various soils, especially under field or field-like conditions.

pH

The results of a number of studies indicate that virus retention by soils generally increases at lower pH levels. In an early report Drewry and Eliassen (14) found decreased bacteriophage T1, T2 and f_2 adsorption to Arkansas and California soils at higher pH levels. More recently, Burge and Enkiri (8) found that the rates of bacteriophage ØX174 adsorption to five soils were significantly correlated with soil pH. In batch adsorption studies with a variety of viruses and nine soils by Goyal and Gerba (25), pH was found to be the single most important soil factor influencing adsorption. Soils having a saturated pH <5 were the best adsorbers. Studies by Sobsey et al. (62) showed that poliovirus type 1 and reovirus type 3 adsorption to eight different soil materials suspended in settled sewage at pH levels between 3.5 and 7.5, was generally greater at the lower pH levels. In studies by Duboise et al. (15) with cores of sandy forest soil receiving poliovirus in sewage effluent at various pH levels between 5.5 and 9.0, virus retention was best at pH 5.5, and the release and migration of retained viruses by subsequent distilled water applications was lower from the cores that received sewage effluent having lower pH values.

Although the mechanism by which pH influences virus adsorption to soils has not been investigated in detail, the effects of pH on virus adsorption to soils are explainable on the basis of electrochemical features of virus and soil surfaces. The surface charge of a virus is influenced primarily by ionization of the carboxyl and amino groups on the outer surface of the virion protein capsid, and at neutral pH, most viruses are negatively charged. Soils also tend to be generally electronegative at neutral pH, and therefore, virus adsorption is not favored due to repulsion of the two negatively charged surfaces. However, if the pH of the surrounding medium is lowered, protonation causes decreased ionization of viron carboxyl groups and increased ionization of amino groups. As a result, viruses become less electronegative or even electropositive at lower pH levels. Although soil particles will also tend to become more electropositive at lower pH levels, the isoelectric points of soil particles are generally lower than those of viruses. Thus, at lower pH levels, the viruses may be electropositive but the soils are still electronegative, thereby resulting in electrostatic attraction and increased adsorption.

Salt Species and Concentrations

The types and concentrations of ionizable salts in the soil-water environment greatly influence the extent of virus transport. In general increasing concentrations of ionic salts and increasing cation valencies enhance virus adsorption.

In an early report, Carlson et al. (9) found that bacteriophage T2 and poliovirus type 1 adsorption to clay suspensions in water was enhanced by low concentrations of $CaCl_2$. The effect was attributed to the role of calcium ions in mediating adsorption between the negatively charged clay and virus surfaces, possibly through clay-cation-virus bridges. Enhanced adsorption of a number of viruses to a variety of soil materials at increased ionic strengths (or concentrations) and in the presence of cations with higher valencies has also been reported by other workers using either soil suspensions or soil columns (14, 42, 58, 62). Recently Taylor et al. (64) reported that pH and the nature and concentration of several simple electrolytes, including $CaCl_2$, NaCl and Na_2SO_4, influences poliovirus type 2 adsorption to 5 soil materials. Virus adsorption from electrolyte solutions was extensive below pH 7 but not above pH 9. The critical pH region where virus adsorption efficiency changed was characteristic for each soil material. Differences in virus uptake among soils were related to their pH-dependent charge properties as measured by microelectrophoresis. The extent of virus adsorption increased with increasing electrolyte concentrations, but only when the pH of the suspension was near or above the critical region for the soil. Poor virus adsorption obtained at high pH was attributed to electrostatic repulsion between the highly negatively charged virus and soil surfaces.

Although solution ions appear to play a role in virus adsorption to soils, the role of exchangeable ions associated with soil particles remains uncertain. In an early report (14) bacteriophage adsorption to nine soils was found to increase with increasing ion-exchange capacity and glycerol retention capacity. More recently Burge and Enkiri (8), using soil suspensions, reported that adsorption rates correlated with cation exchange capacity for four of the five soils tested. However, Goyal and Gerba (25) found that the adsorption of a number of different viruses to nine different soils was not significantly correlated with cation exchange capacity, total phosphorous, total and exchangeable iron and exchangeable magnesium.

Decreases in salt concentration or ionic strength can cause adsorbed viruses to be released from soils and then be transported more extensively. Under simulated cycles of intermittant effluent application and rainfall (distilled water application) to cores of sandy forest soil, the extent of poliovirus migration depended on ionic gradients produced in the waters as they passed through the cores. Applications of distilled water caused decreases in ionic strength of the column effluents and associated increases in virus elution. Virus elution also coincided with increased elution of total organic carbon, color and turbidity. Similar findings have also been reported by other workers (20, 39, 62).

The migration of poliovirus in soil columns under changing ionic conditions has also been studied by Lance et al. (37). When poliovirus type 1 in secondary effluent was applied to columns of calcareous sand, most of the viruses were retained in the upper five centimeters of soil, regardless of the infiltration rate. When deionized water was subsequently applied, viruses desorbed from the soil and migrated further down the columns. However, when this was followed by another application of sewage effluent, the viruses were readsorbed by the soils further down the column and they did not appear in column effluents. These findings suggest that virus movement through soils as a result of heavy rains may be minimized by appropriate management of flooding and drying cycles in rapid infiltration systems.

The results of a number of studies indicate that virus transport through soils is greatly influenced by ionic conditions of the soil-water environment, and they provide a basis for explaining the sudden appearance of natural virus contaminants in groundwaters following heavy rains at field sites where wastewaters are being applied to the land (68). However, considerably more field work is needed with a variety of soil types, climatic conditions and wastewater application systems to better establish the extent of virus movement in soils under changing ionic conditions.

Organic Matter

At least certain types of soluble organic matter in water and soil will decrease virus adsorption to soils. In an early report Carlson et al. (9) found that proteinaceous organic matter interfered with bacteriophage T2 adsorption to three types of clay suspended in $CaCl_2$ solutions.

Humic and fulvic acids are highly colored organic compounds that are naturally present in both water and soils (60). Recent studies indicate that these compounds can cause increased virus transport through soils not only by interfering with virus adsorption but also by causing desorption. Bitton et al. (5) found that poliovirus retention by columns of sandy soil was extensively reduced when applied in highly colored (high concentrations of humic and fulvic acids) cypress dome water compared to its retention from tap water. More recently, Scheuerman et al. (59) reported extensive interference by humic and fulvic acids with poliovirus type 1 retention in columns of organic sediment, muck soil and brown-red sand. Soils that were capable of retaining all or most of the applied virus in the absence of these organics retained considerably less virus in their presence. The extent of virus transport through the columns correlated with the color of the column effluents.

In batch studies, Bixby and O'Brien (6) found that fulvic acids complexed with bacteriophage MS2 and extensively interfered with its adsorption to a fine loamy sand.

There is conflicting information in the literature concerning the ability of wastewater organics to interfere with virus retention by soils. In an early study by Carlson et al. (9) bacteriophage T2 adsorption to kaolinite was somewhat reduced when the suspensions contained as much as 10 mg/ℓ of sewage. More recently, Moore et al. (48, 49) reported that certain microbially produced wastewater organics interfered with virus adsorption to soil materials. Bacterial exopolymers from *Zoogloea ramigera* greatly reduced reovirus adsorption to 3 sand size soil materials (beach sand, Ottawa sand and magnetite) and protected against the loss of virus infectivity associated with adsorption. However, indigenous wastewater microbial exopolymers in primary sewage effluent were less effective in preventing adsorption and protecting against inactivation.

In contrast, Gerba and Lance (20) found that poliovirus removal from primary and secondary sewage effluent in columns of loamy sand was similar for both types of wastewater.

The results of a number of studies suggest that organic soils and other soils or waters with high concentrations of humic and fulvic acids may not be suitable for land application of wastewater. The effects of other organics in waters and soils on virus retention remain uncertain. Additional studies are needed to further understand and quantify the effects of humic and fulvic acids in water and soil on the infectivity and retention of a variety of viruses in different soils. Such studies are also needed for other classes of water, wastewater and soil organics.

Hydraulic Conditions and Moisture Content

Hydraulic conditions in soils receiving wastewater appear to have a considerable effect on virus transport, for at least some soils. Such conditions as flow rate, hydraulic loading and application frequency may all influence the extent of virus migration through soils.

In an early report Robeck et al. (54) found that the extent of poliovirus removal from tap water applied to a sand column decreased with increasing flow rates above 1.2 meters per day. Recently, Vaughn et al. (67) reported that infiltration rate greatly influenced poliovirus removal in a groundwater recharge system where tertiary effluent was applied to a coarse sand-fine gravel soil. Recharge at 75-100 cm per hr. resulted in considerable virus movement into groundwater while at two lower recharge rates, 6 and 0.5-1.0 cm per hour, there was considerably less virus movement. At the lower infiltration rates, the surface mat of sewage solids that formed on the soil surface may have contributed to the greater virus removals observed.

Using sand columns dosed with poliovirus in septic tank effluent, Green and Cliver (26) found virus retention to be much lower under saturated flow conditions created by high hydraulic loadings compared to those under unsaturated flow conditions at lower hydraulic loadings.

Somewhat in contrast to these findings are those of Lance et al. (37) and Lance and Gerba (36) who studied poliovirus movement through a coarse sand column flooded with sewage effluent at a high rate. In an early study Lance et al. (37) found that poliovirus type 1 removal was not affected by infiltration rates in the range of 15-55 cm per day. More recently Lance and Gerba (36) found that increasing flow rates from 0.6 to 1.2 meters per day resulted in increased movement of viruses down the column. However, there was no further increase in virus movement at flow rates up to 12 meters per day. They concluded that virus retention by soils is increased above some breakpoint flow rate, but flow rate changes above and below this breakpoint do not affect the extent of virus retention.

Although the results of at least some studies suggest that virus migration increases with increasing hydraulic loads and flow rates and under conditions of saturated flow, further studies are needed with a wide range of soil types and field conditions to quantify the extent of virus movement through soils under different hydraulic conditions.

Summary and Conclusions

This brief review has shown that a variety of factors influence the transport and survival of viruses in soils. A major factor influencing virus survival is temperature, with generally increased survival at lower temperatures. Soil moisture also influences virus survival, with decreasing survival in dry or drying soils. However, information in the literature is not entirely consistent regarding the moisture levels at which maximum virus inactivation occurs, and more studies are needed in this area with a variety of viruses and soils. Aerobic microbial activity also contributes to virus inactivation in soils at moderate to high temperatures. Further research is needed to precisely identify what is responsible for microbially-mediated virus inactivation in soils and to determine the relationships between microbial activity levels and virus inactivation rates.

Although pH and ionic effects may play a role in virus inactivation in soils, these properties are related to the chemical and mineralogical characteristics of soils. Information in the literature is not entirely consistent on which chemical, mineralogical and textural properties of soils are most influential upon virus survival or inactivation. It may be possible to predict relative survival of viruses in different soils, based upon their specific properties, but further studies are needed in this area.

Viruses differ in their ability to survive in soils, and more viruses must be evaluated to determine the overall range of virus survival. Such studies are especially needed with those viruses that have been responsible for outbreaks of disease associated with contaminated soil and groundwater, such as hepatitis A virus and the Norwalk-type gastroenteritis viruses.

The role of organic matter on virus survival in soils is uncertain. Although interaction with humic and fulvic acids may cause reversible loss of virus infectivity, there is also some evidence that such virus complexation with organics may actually protect viruses from inactivation by preventing their adsorption to soil particles. The latter effects may result in greater virus survival and mobility in soils. The effect of organic matter on virus survival in soils clearly needs further investigation.

Virus retention by soils is also influenced by a variety of factors, some of which also influence virus survival. Virus association with particulate matter may enhance retention in soils but may also result in longer survival. The net result of these two effects remains uncertain, especially under field conditions. Soil type also influences virus retention, and adsorption is an important mechanism of virus removal in soils. Although virus retention is generally lower in sand and organic soils than in clay soils, information in the literature is inconsistent on which specific soil properties are most important in virus retention. Further research is needed in this area, especially under field or field-like conditions.

Viruses differ in their ability to be retained by soils and more viruses must be evaluated, especially indigenous viruses in water, wastewater and sludges.

Ionic conditions and pH also influence virus retention by soils and retention is generally better at lower pH levels and at high salt concentrations or with salts having higher cation valencies. The effects of these factors are also related to the characteristics of the soil material and other environmental factors such as hydraulic conditions and rainfall. Therefore, they must be evaluated for a variety of soils under a range of conditions encountered in the field.

As previously indicated, humic and fulvic acids interfere with virus retention by soils and therefore, waters and soils with high concentrations of these organics may be unsuitable for land application of wastewater. The effects of other organics on virus retention by soils remains uncertain. Additional studies are needed to determine the effects of humic and fulvic acids and other organics on both the transport and fate of viruses in soils, especially under field conditions.

Increased hydraulic loads and flow rates may increase virus transport in soils, especially when the soils become saturated. However, additional field studies are needed with a wide range of soils to quantify the extent of virus movement through soils under different hydraulic conditions.

Although a variety of factors that influence virus transport and fate in soils have been identified, there is as yet insufficient information in the scientific literature to accurately predict the extent to which viruses will travel or persist in the soil environment of a particular field site receiving virus-contaminated water or waste. Such predictive information is a desirable objective for future research efforts.

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TRANSPORT AND FATE - VIRUSES IN SOIL: MARK SOBSEY

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: Lennette

Mark, you mentioned something which I think might have been overlooked, because you went so fast, and that is the characteristics of LSC 1 polio virus. Its properties are such that some people question whether this strain is a useful model for transport and similar studies. You could amplify this point I think. Strain properties are very important, and the environmental virologist might take a page out of the medical virologist's book and characterize the strains with which he works. What is Echo 13 in one laboratory may not be the same as an Echo 13 in another. The history and animal and cell culture records unfortunately in most cases have been inadequately kept so that no one knows the real passage histories of many of these strains. The kind of animals and the kind of cell cultures in which these strains have been passaged, how many passages have been made in any one animal species or in any one type of cell is important because these factors give rise to a selection or mutation process(es) which endow the strain with the properties or genetic markers that characterize it. Polio virus type 2 seems to be the pet model of the water virologist, but frequently no distinction is made as to the strain employed so that its pathogenic properties might be given due weight. A comparable situation exists in cell biology and in the cancer field where many investigations represent a questionable or wasted effort because of failure to check or monitor the cell line or cell strain being used. Great care is required to prevent contamination of one cell line with another, say a human cell line with a mouse line. In this connection, a human carcinoma cell, the well known Hela line, is notorious for the ease with which it is picked up in a laboratory so that unless the chromosomal markers characteristic of a given cell line or strain are periodically checked, an investigator who thinks he may be working with the line in which he started his experiments originally may well be working with a Hela line contaminant. Furthermore, when Hela lines are used, they are not necessarily identical in their properties from laboratory to laboratory.

I would now like to speak as an epidemiologist and an infectious disease physician, not to my peers but to the engineers and chemists and toxicologists and pharmacologists in this audience. One of the tabulations you will see in paper after paper is a listing of hundreds of viruses, or at least scores of viruses, that are found, or may be found, in wastewaters. Unless one is very close to the infectious disease field, the natural inference is that all of these agents pose a disease hazard. Factually, this is misleading and some qualification is necessary. Jim, you presented several tables in a context somewhat different from what I am discussing here, and no criticism is intended, but I should like to borrow your Table 1 as an example of the multitude of tables which appear in many published papers. Thus, the presence of mycobacteria in water has no significant role in the pathogenesis of tuberculosis. Tuberculosis is a respiratory disease acquired through the respiratory tract as a portal of entry. Similarly, poliomyelitis cannot be construed to be a waterborne disease merely because the virus may be present in water. If you will go back to the early literature on this subject -- the trouble with some so many investigators nowadays is that they do not go back to the early records; anything that is more than five or ten years old is overlooked -- if you will go back to the 1890's or so you will find that Wickman in Sweden described what at that time was called infantile paralysis, and which was assumed by him and other epidemiologists to be a waterborne disease because of it was along rivers. However, in the light of present knowledge, we know that this assumption was erroneous, because Scandinavian rivers provided the major means of transportation and the great preponderance of settlements was along the rivers. There is no evidence today that poliomyelitis is a water-produced disease. Pathogenic agents present in these waters are not necessarily going to be a hazard. What is present in these waters is a reflection of what is being excreted in the community, animal and human. Thus, to repeat, the mere presence of a pathogenic agent in wastewater does not necessarily imply that it plays a role in waterborne disease. However, I am anticipating what Vic Cabelli is going to say, and, I guess, Ricky Wilson and Elmer Akin also, so I will just leave the rest of this aspect for them to discuss tomorrow.

I could not resist inserting this caveat because there are so many misapprehensions when people look at tables such as I mentioned. For example, just because adenoviruses are found in water doesn't mean that they produce disease; I am talking about drinking water and wastewater. Adenoviruses in large part produce respiratory disease, yet the best way to isolate the virus for diagnosis is not from the throat but from a stool specimen from the patient. Similarly, in attempting to immunize people against adenoviral infection, the best approach is to give the virus orally in enteric-coated capsules in order to permit the virus to colonize the gut. This illustrates how necessary it is to know the pathogenesis, immunology and epidemiology of a disease before one can characterize, in any given situation, an agent as an actual or as a potential hazard to health. So my caveat is "do not take literally all of the listings you see of these viruses as being known hazards to man".

A: Sobsey

I think Dr. Lennette's point should be well taken. Although we can list over 100 different viruses that are partially present in human feces and in fecally contaminated water, rarely, I suspect, would they ever be all present at the same time. One of the great enigmas in this area of research is that the viruses that are apparently the most imoprtant in causing waterborne outbreaks, hepatitis type A and acute gastrointeritis viruses, are extraordinarily difficult to cultivate in the laboratory. Therefore, we have been unable to work with them, and it will require some breakthroughs in the cultivation of these viruses before they can be examined in the types of water, wastewater, and soil studies that have been done already with viruses, such as polioviruses and echoviruses. It is unfortunate that those studies can't be done, because information is needed on the detection, occurrence, survival, and fate of these non-cultivatable viruses in the environment. I think Dr. Lennette's point about virus strain differences is quite important. Recent studies by Vaughn, Landry and colleagues, and Gerba and Goyal and colleagues, point out that there are dramatic differences in the behavior of the same virus type, depending upon which strain is used. One can take several different strains of the same virus, do experiments on virus adsorption to soils and show dramatic differences in the extent of virus retention. I think this should make us aware of the risks involved in using a single virus or even just a few selected laboratory grown viruses to obtain definitive information on how all entroviruses are going to behave in a soil environment.

Q: Morrison

Mark, I was trying to put together a question in terms of your factors relating to survival, destruction, retention, and so forth, of the viruses. Both your work and the review articles and the real live world of the epidemiology of disease transmission, and Dr. Lennette I think framed the question well. I would like to add a plea to the epidemiologist that we need a refinement of our epidemiologic observations and quicker response so that the things that you are doing, that is the source of the infection and the particular factors that may have contributed to the disease incident can better be understood. Then we can better interpret why at some point in time in a sewage treatment outfall or recreation water or drinking water we go along for years with no disease and all of a sudden an infection breaks out. We haven't refined our epidemiology adequately.

A: Sobsey

Perhaps some of the missing links here between the occurrence of viruses in water and soils and the occurrence of waterborne disease will be brought together at this conference. I think that this is one of the great difficulties in this field. Many of us spend a great deal of time in the laboratory and in the field looking for viruses in contaminated water and soil and doing studies to determine the extent of virus movement and survival in the environment. Unfortunately, we do that out of context to the exposure of individuals to virus laden water or other materials. On the other hand, common source enteric viral disease outbreaks due to contaminated water and contaminated foods continue to be reported. Some of those outbreaks have occurred in situations where indicator bacteria levels were quite low, and this has raised considerable concerns about the suitability of bacterial indicators. Unfortunately, we have not been very successful in linking the epidemiological information from outbreaks with the information from studies on virus transport and fate of the environment. Perhaps in some of the later papers during this conference when epidemiological considerations are discussed in more detail, risk assessment approaches to determine the significance of viruses in the soil and ground water environment will be established.

Q: Lance

You mentioned some conflicts in data with respect to moisture content, that is, the effect of soil moisture content on viruses. I think part of the problem is that we must separate direct effects from indirect effects. The direct effect of soil moisture on virus survival probably doesn't occur until the soil is dried to a 2 percent moisture content, but you have indirect effects before the soil dries to that level. As the soil dries, this allows the growth of the aerobic bacteria that are antagonistic to viruses. If you saturate a soil and maintain that moisture level, you never will get aerobic bacteria. Therefore, soil moisture indirectly affects virus survival due to its effect on the soil oxygen content that in turn affects the population of aerobic bacteria. Secondly, to make the conference a little more interesting, let us interject a little disagreement. Let me disagree with you about differences in soil adsorption among different virus strains. I have yet to see data from any source that approximates field conditions and shows differences in the adsorption of different virus strains by soils. We were aware that a number of studies showed differences in adsorption among different virus strains when batch studies or very short columns were used and we tried to follow up on that. We couldn't find any differences between the adsorption of virus strains by a soil column that closely approximates a field system. The same soil and the same virus strains that showed adsorption differences in batch studies did not show those differences in the soil column. So I think we want to be sure that we don't get sidetracked by differences that may be due to our laboratory procedures. The final thing I want to point out is: we can sit through a day like this thinking about what we know about viruses and become discouraged. Some may leave the conference and say, "Boy, we are in a terrible condition because we don't know anything." Well, we do know some things. We can't predict how far in every circumstance that viruses will move, but we can develop a system where they don't move. We can develop a system that has shown to effectively limit virus movements. For example, if you have a 10-ft. depth of a soil without channels, it is very difficult to move viruses through that layer of soil. Even if you choose a gravelly soil, you can regulate the infiltration rate so that very few viruses move through the soil. Therefore, we do know how to put an effective block between the soil surface and the ground water; although we can't predict exactly how far in that 10 feet of soil that viruses will move.

A: Sobsey

Well, I believe it is possible with engineered soil systems to manipulate them in such a way to optimize virus removals. I think it would be useful though to be able to look at a natural site that may be proposed for land application of wastewater, whether it be a large scale system or a septic waste disposal system, and examine the soil and hydraulic conditions at that site in order to estimate or predict the extent to which viruses will move. This is an area where we will still have difficulty. It is now difficult to examine the textural, chemical and mineralogical properties of soils and the hydraulic conditions of a land application site and be able to make definitive statements about the extent to which viruses will move. Although we can manipulate some soil systems to get more unsaturated flow and distribute the waste over a larger area to improve virus retention and the retention of other pathogens, we still need to do more to try and develop better predictive tools.

So far as the type and strain dependence of viruses on their behavior in soils, it is true that a lot of the studies done so far have been small laboratory batch suspension studies, or small column studies, and there is always some danger in extrapolating from that information to field conditions. However, I think that there are sufficient differences to warrant further investigation of type- and strain-dependent virus differences with respect to their movement under field or field-like conditions. I, for one, would like to see those experiments done.

Q: Lennette

These several strain differences to which Dr. Hagedorn referred have not been susceptible of analysis. As you may know, virology is really the foundation on which molecular biology has been built, and in return the molecular biologist has given the virologist some tools to approach these problems. One of these is fragmentation of the viral genome by means of enzymes produced by bacteria. The various enzymes split the DNA at different points at intervals of so many kilo bases or even so many hundred bases. It is possible, by putting the resulting fragments on an agarose gel and using electrophoresis to separate them out by size. By matching the fragment sizes from one viral strain with those of another, it is possible to determine whether the two strains are identical or whether they are different. This provides the epidemiologist with a tool to study outbreaks of, for example, herpes virus infection and to follow the spread of the virus from patient to patient, and to determine and follow the spread of nosocomial infections.

Another approach, one which is creating much interest and enthusiasm in biology and in immunology specifically is the hybridoma and monoclonal antibodies. For example, if one inoculates a mouse with an antigen, whether that antigen be a bacterium or a virus or a protozoan or whatever, the animal produces a whole host of antibodies to the various antigenic fractions which compromise that bacterium or virus or protozoan. The immune serum, in effect, contains a mixture of antibodies which cannot be separated out and hence the serum has a very broad reactivity.

If instead of letting the antibody-producing cells in the spleen pour out antibodies into the general circulation, we remove the spleen, separate out the antibody-producing cells and fuse them with myeloma cells, we have a means of separating the various antibodies. The myeloma cell, which is a blood cell tumor, is immortal and multiples rapidly. The hybrid of the myeloma cell and the antibody-producing cell, called a hybridoma, is thus a rapidly multiplying cell which produces antibody in large amounts in this rapidly replicating system. Various clones of these cells can be produced and sorted out to obtain those which produce a single specific antibody. Such antibodies can be tagged in a variety of ways, for example, with a fluorescent dye or with a radiolabel and provide a means for identifying various components of the virus and thus picking up strain differences.

Finally, let me say that what I am hearing at this conference today has a quite different tenor from what I heard five years ago, in that investigators today are considerably more cautious aobut what they are saying.

Q: Gregg

It seems to me that one of the critical factors that I see in all of what we have been saying today is the tremendous need to study what is naturally occurring in our environment. I think that it is all well and good to look in the laboratory and we learn of determinants and we learn of various areas that need to be studied, but I would make a strong plea for a major interdisciplinary study of that which is occurring all of the time. The natural experiments of nature if you will. We can take, I think, some benefit from our colleagues who have studied arboviruses where if you just study human diseases that are caused by mosquitos and fail to look at what is going on in the mosquito population or in the bird or animal population you don't really learn very much. So I think that we need to bring disciplines together. We need to bring people together as we are doing today, indeed. But to consider very strongly the intensive investigation of outbreaks from a variety of points of view to try to understand what the factors were that contributed and produced human disease.

Q: Wilson

I would like to point out that it is not just a matter of studying the outbreaks, but it is studying things prospectively Allusions have been made to water samples with low fecal coliform counts, outbreaks of viral illnesses, and I think it is important to point out that we are usually retrospectively studying the water systems after the outbreak has been recognized. There is usually a delay in that recognition so that I think that I would put in an even stronger plea that we have to have a plan so to speak ready to have early intervention if you are really going to put all of the pieces of the puzzle together at the same time. One of our great handicaps in epidemiology is that we are going in after-the-fact-fashion, trying to piece together what has already happened, and then it is often very difficult to correlate water system, human disease, etc., in one coherent fashion in all cases because we are dealing with different time frames.

Q: Gregg

I'm sorry, I should have emphasized surveillance; this is terribly important. The prospective study is indeed important and this is what the arbovirologists have done. They start collecting specimens in the early spring from birds and from animals and they start surveillance of human disease. This gives them at least a much better handle on the dynamics of what produces disease and I just want to emphasize what Dr. Wilson said.

Q: Cabelli

I would like to pursue one of the points Dr. Sobsey made. In response to one of Dr. Lennette's comments, he noted that the technology is as yet unavailable for cultivating the viruses of most apparent concern as seen from the epidemiological record, hepatitis A and the agents of acute gastroenteritis. This is unfortunate. A more regrettable fact is that we have spent and continue to spend large sums of money on developing data bases for the enteroviruses, although there are scant epidemiological data to support their importance via waterborne routes of transmission. More regrettable yet are the notoriety consequent to the recovery of even a single enteroviral PFU from extremely large quantities of environmental waters and the efforts to promulgate standards for these agents.

I also wonder about the reality of transport, survival, and/or adsorption data produced with tissue culture-grown enteroviruses, even when they are mixed into sewage. The reported differences in survival and adsorption of different viruses may not be as great when the virions are naturally present in the sewage. The rub, as we all know, is that "heroic" concentration methods are required to recover the naturally occurring enteroviruses from environmental waters and that the accuracy and precision of these methods leave something to be desired. It seems to me that equally, if not more, valid data can be obtained from the bacteriophages found in sewage

-- especially the male-specific phages -- and that these data will be considerably less expensive to produce.

My second concern is that all too many individuals think of coliforms and fecal indicators as being synonomous. In fact, there are a number of health effects, water quality indicators, and, for most if not all applications, one or more of the other systems is more appropriate than the coliforms systems.

It seems to me that the data from transport studies, whether in surface waters, soil or ground waters, would be more easily interpreted if an appropriate conservative tracer were used to separate physical removal from biological decay.

A: Sobsey

I think that Dr. Cabelli's point about perhaps having spent a lot of time studying the wrong viruses is well taken. Particularly if some of the viruses that we should be most concerned about are present at perhaps higher concentrations than we might suspect. This may be the case with rotaviruses. It is not clear whether it is true of such viruses as the Norwalk agents or the hepatitis A virus. The latter do not appear to be excreted in particularly high concentrations. There are no end to the surprises in this field. For example, a lot of people cite the 1955-56 New Delhi epidemic of infectious hepatitis as a classical significant waterborne viral disease outbreak. It turns out that, in fact, the outbreak was not due to the hepatitis type A virus that we know. It is due to some other fecally excreted epidemic hepatitis virus that hasn't even been isolated and characterized yet. The evidence for this has only recently become available. There are clearly many more things that we need to learn about waterborne viruses and their impact upon public health.

Q: Lennette

This is another ghost that ought to be laid sometime because the New Delhi epidemic is referred to in the water-virus literature _ad nauseam_ in my opinion. What was involved was not drinking water that was properly treated; it as water that was about half sewage. So you could expect to have an outbreak.

Q: Akin

I wanted to make just one comment. Thank you Dr. Lennette for opening it up tonight. We had you on the panel because we knew you had a bias and we knew you would be willing to share it with us and I am very appreciative of this last discussion. We quit being nice guys now; we can really get down to the heart of the matter. We criticize and we can deal with these issues tomorrow as we are beginning to this evening. We who work in this field are frustrated. We know there are a hundred viruses that can be shared through the intestinal route and we know there is very little evidence for disease transmission of very many of these. We also know, as Dr. Rickey Wilson is going to tell us tomorrow, that you can have an outbreak of waterborne disease where 79 percent of a community can be infected with a virus. Why does that occur in that situation and not occur in others? I think there are many unknown questions that we want to deal with. We are, I think, frustrated in a sense that we know that sewage can contaminate a water supply. We can drink a glass of that water, it won't be discolored, it won't have an odor, so therefore the contamination must be relatively low, but yet a large percent of the population can be infected by that. Polio virus is in stool, it is in all of the sewage samples any virologist samples, but yet there is not good evidence that polio as you said was ever waterborne, and I think that is an enigma that has lived with us for 20 years now without a good answer. Why is that the case? So I think tomorrow as we delve into some of the other aspects we will have an interesting day. I am sure none of us are envious of the panelists' job. Mark had 79 references in his paper. There is a lot known but I couldn't help, Mark, when you were talking to say, "Gee, I almost share what some senator said, 'I wish scientists only had one arm because they are always saying on the other hand.'" On the other hand, we never really get to a bottom line do we? This data shows this but here's something else that shows something else. So it is a frustration and I hope as we discuss and the panel submits its report that we can get to the bottom line on some of these areas.

THE LATERAL MOVEMENT OF INDIGENOUS ENTEROVIRUSES IN A SANDY SOLE-SOURCE AQUIFER

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ABSTRACT

Several studies conducted during the last ten years have identified the potential for virus movement through soil horizons and into shallow ground water systems. Few studies to date have provided evidence for lateral movement of ground water entrained viruses. The study described herein focused on lateral human virus movement from a subsurface wastewater disposal system through a shallow, sandy soil aquifer. Minimal virus movement was confirmed at an observation well located 45.7 m from the septic source. Preliminary data indicated virus movement through an additional 15 m of the aquifer (60 m from the septic source). The data indicated the potential for significant lateral entrainment of viable human viruses through an aquifer. Virus occurrence could not be correlated with that of standard bacterial water quality indicators.

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INTRODUCTION

The past several years have witnessed the development of considerable information on the ability of human viruses to penetrate soil horizons and contaminate groundwater systems(1-5). Given the possibility of viral contamination of an aquifer by sewage disposal (e.g. subsurface disposal systems, wastewater recharge basins), major considerations must be given to a determination of the extent of viral entrainment and lateral movement in groundwater. The need for such information is particularly acute in areas, such as Long Island, where sewage-contaminated aquifers serve as the sole source of potable water for the local inhabitants.

Over the past decade, several authors have presented direct and indirect evidence for significant virus movement through groundwater. Mack et al.(6) isolated poliovirus type 2 from a 30.5 m deep drinking water well located some 91.5 m from the edge of a wastewater drain field. Similar findings were reported by Wellings(7), who described soil penetration and lateral movement of viruses through groundwater following the discharge of secondarily-treated wastewater in a cypress dome. In a later study, Wellings et al.(8) recovered echovirus 22/23 from a well located thirty lateral meters from a wastewater disposal site. Vaughn et al.(3) reported sporadic isolations of enteroviruses from two rapid wastewater infiltration sites located on Long Island. At the first site, echovirus type 12 and several unidentifiable isolates were recovered from a groundwater observation well adjacent (3.0 m) to a sand recharge basin receiving secondarily-treated wastewater. Virus recoveries at the second site provided additional evidence for lateral movement in the aquifer. Here, the observation well was located 45.7 m down-gradient from sand basins receiving tertiary wastewater effluents. Schaub and Sorber(9) reported virus isolations in groundwater beneath rapid infiltration units receiving secondary effluents. Viruses were recovered at depths of 30 m, and at lateral distances of 182.8 m. Koerner and Haws(10) observed the results of the rapid infiltration of primary effluent through coarse sand and gravel. Polio-, coxsackie- and echoviruses were recovered from groundwater at a depth of 16.8 m. The authors also reported virus isolations at 250 m down-gradient distances.

Several studies have specifically focused on the vertical and lateral movement of viruses from subsurface disposal systems. Vaughn and Landry (11) isolated unidentifiable virus types from a groundwater observation well located 3.3 m down-gradient from a subsurface leaching pool receiving secondarily-treated effluents. Wellings et al.(8) demonstrated the presence of echovirus 22/23 in a series of groundwater wells clustered in an area surrounded by septic tanks. A similar virus source was proposed by Vaughn and Landry(11) following the isolation of echovirus types 11 and

23, and coxsackie A-16 from a groundwater observation well located in the center of a cluster of single-family dwellings which discharged wastes to individual septic systems.

Epidemiological investigations of waterborne disease outbreaks have also provided evidence for the lateral transport of viruses from septic sources. Vilim et al.(12) reported on an outbreak of waterborne hepatitis in a small Czechoslovakian community caused by subsurface wastewater penetration of a nearby drinking water well. Two hepatitis outbreaks, occurring in Polk County, Arkansas, were attributed to the consumption of groundwater contaminated by lateral drainage of leachates from septic tanks(13). Gastrointestinal outbreaks involving 759 persons in Missouri and Arkansas, in which no bacterial etiological agent could be isolated, were associated with consumption of groundwater from wells near municipal oxidation lagoons. Investigators suggested that leachate from the lagoons had percolated through porous limestone and contaminated the groundwater(14).

The present study (funded by the Suffolk County Department of Health Services) was designed to assess the lateral transport of indigenous human viruses through a shallow sole-source aquifer receiving discharges from a septic system. The study focused on the likely contribution of subsurface disposal systems, set in sandy-gravel soil, to shallow aquifer contamination, and their potential impact on nearby drinking water supplies.

MATERIALS AND METHODS

Site Selection and Well Installation

The site chosen for the study was the Hampton Gateway Apartment complex, Speonk, New York, located on the South Shore of Eastern Long Island. This site was chosen from among several candidate locations on the basis of the following considerations: 1) The site contained several multi-family dwellings served by a single subsurface waste disposal system which recharged a significant amount of septage leachate to the Upper Glacial aquifer; 2) The up-gradient area of the site was largely undeveloped woodland, with no significant input of human virus to the aquifer. Isolations of human viruses from the septage-influenced groundwater at the study site could reasonably be associated with the disposal system under study; 3) The subsurface disposal system was installed in an open field, which facilitated the placement of wells down-gradient from the septage source. It also provided convenient access for sampling; and 4) The site was located some 25 kilometers from Brookhaven Laboratory, a factor which greatly facilitated the rapid processing of samples.

The apartment complex consisted of three buildings, each containing 40 apartment units. Since the original plans had called for the eventual construction of three additional buildings (80 units), the disposal system

(Figure 1) had been designed to accommodate six structures serving approximately 300 people. The system consisted of a 57,000 liter septic tank, three distribution pools (4.25 m^3) and 40 leaching pools (4.95 m^3). Each pool was installed approximately 3 m below grade, which resulted in a 3.6 m unsaturated zone above the static groundwater level. As the system operated at approximately one-half capacity, peripheral pools received little flow from the central septic tank. The major leachate plume was found to be concentrated around the central pools. Plume and groundwater-gradient measurements were conducted by the Groundwater Resources Section of the Suffolk County Department of Health Services. Their investigations showed that the groundwater flow was South 40° West, with an hydraulic gradient of 2.22 m per mile (Craig Werle, personal communication). These measurements were in general agreement with the local hydraulic gradient shown on regional water table maps. The configuration of the septage percolate plume was determined on the basis of physical and chemical measurements made on groundwater samples taken from eight test wells driven down-gradient from the leaching pools. Measurements included conductivity, ammonia, nitrate, nitrite and chloride determinations. An additional well was driven northeast (34.6 m) of the leaching pool array to serve as an uncontaminated groundwater control. The measurements indicated that the septage percolate was concentrated down-gradient from the center of the sewage disposal system, moving slightly away from the hydraulic gradient. On the basis of these examinations, a series of monitoring wells were driven along the line of predicted plume movement. Using the center of the system as the point of origin, wells (5.08 cm diameter) were driven at distances of 1.52, 3.05, 4.57, 7.62, 10.67, 15.24, 22.86, 30.48, 45.72 and 60.35 m respectively. Each well was fitted with steel piping having a 0.91 m stainless steel screen section set 1.52 to 1.83 m below the top of the water table.

As seen in Figure 1, a sewer line serving building #3 crossed the study area between the 15.24 and 22.86 m monitoring wells. Since any virus isolations from these wells could be questioned on the basis of possible leaking from cracks or defective joints, the Health Department provided a detailed television inspection of the sewer line to determine its status. In addition to optical inspection, each joint was individually pressure-tested (testing conducted by PENGAT Contracting Corporation, Setauket, New York). All joints in the vicinity of the testing wells were found to be intact. Inspection of the entire pipe indicated no other defects at any point.

Field Sampling

Sample Collection

Groundwater samples (380 ℓ) were collected from each sample well at bi-weekly intervals during the initial phase of the study. Samples were collected in 209 ℓ acid-sterilized tanks (Plasti-i-Cube, Grief Brothers Corp.) which had been arranged in permanent sets for exclusive use at each sample well. Pumping equipment and hosing (also arranged in sets for certain well groupings) were acid-sterilized before each collection and

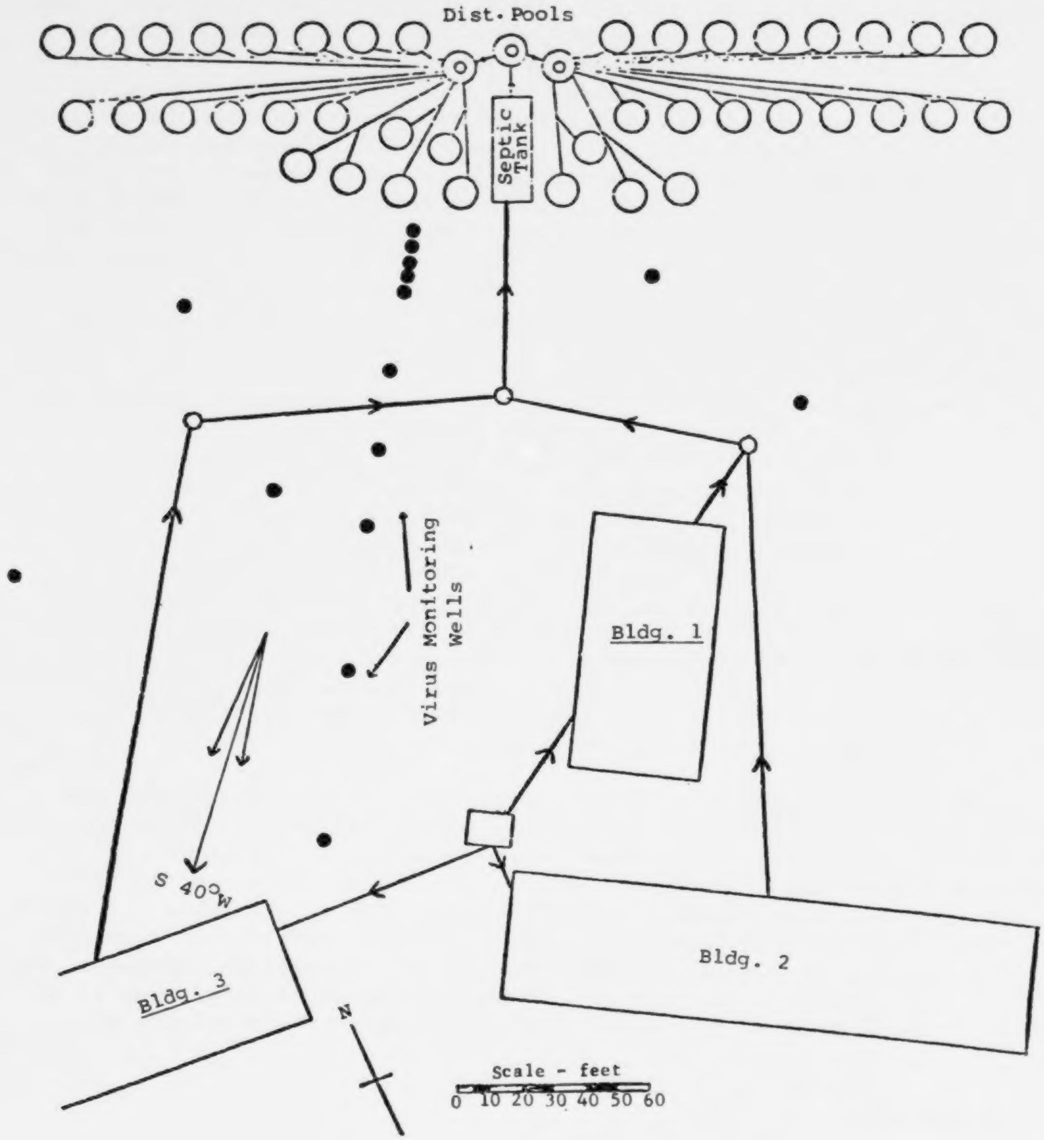
Dist. Pools
Septic Tank
Virus Monitoring Wells
Bldg. 1
Bldg. 2
Bldg. 3
S 40° W
N
Scale - feet
0 10 20 30 40 50 60

Figure 1. Monitoring Well Locations.

thoroughly rinsed with 50-100 ℓ of sample water prior to the filling of the tanks. Bi-weekly, 38 ℓ samples were also collected from the central septage distribution pool in order to approximate input levels. Septage samples were also collected in containers used exclusively for this sample type. The above measures (plus others described below) were undertaken to avoid the likelihood of cross-contamination between different sample types.

Virus Concentration and Enumeration

Concentration of viruses from large volume samples involved a filter adsorption - beef extract elution - organic flocculation method(15). Briefly, samples were acidified to pH 3.5, supplemented with 0.5 mM $AlCl_3$ and passed through a virus concentrating filter series consisting of a fiberglass depth cartridge filter (K-27) and a pleated cartridge filter (Duo-Fine, Timonium, Md.). Viruses were eluted from concentrating filters with 1-ℓ volumes of 3% beef extract - 0.05 M tris (hydroxymethyl) aminomethane (pH 9.5). Virus reconcentrations from 1-ℓ eluates followed the technique of Katzenelson _et al._(16). Eluate pH was adjusted to 3.5, causing the formation of a virus-adsorbing protein precipitate. After 30 min, the precipitate was collected by centrifugation (5000 x g/10 min) and the resulting pellet dissolved in 20 to 25 ml volumes of 0.15 M dibasic sodium phosphate (pH 9.0). Concentrates were then neutralized to pH 7.2 and stored at -60°C to await assay.

Viruses were enumerated on monolayers of low-passaged Buffalo Green Monkey kidney cells (BGM) which had been propagated in Eagle's Minimum Essential Medium with Earle's balanced salt solution supplemented with 10% calf serum, glutamine and antibiotics (penicillin, streptomycin and gentamycin). Sample concentrates were diluted (1:3), inoculated (4 ml) on BGM monolayers in 75 cm^2 flasks, and incubated with rocking for two hours to facilitate virus adsorption. After decanting excess inoculum, monolayers were overlain with 15 ml of MEM plaquing medium supplemented with 2% calf serum, agar and antibiotics(17,18). Flasks were incubated 1-3 days at 36° C under 5% CO_2, and then stained with a second overlay (5 ml) containing agar and neutral red. Flasks were returned to the incubator and observed over a 10-day period for plaque development. All plaques were confirmed by two passages on cell monolayers. Only samples showing consistent cytopathic effects (CPE) were considered positive. Because of the relatively low virus numbers expected, entire sample concentrates were assayed.

Virus concentration units were acid-sterilized between each sample processing. Two separate units were constructed for use in the study, one for the "near well" samples (1.52 to 10.67 m wells), the other for the "far well" samples (45.72 to 60.35 m and control wells [northeast well]). The relative efficiencies of both units were tested prior to initiation of the sampling program using poliovirus-seeded groundwater collected from various study wells. Recovery efficiencies for both units ranged between 65 and 87%. Seeded groundwater experiments were also conducted to compare the relative recovery efficiencies of the described filter series versus electro-positive filters (Zeta-plus) provided by the AMF Cuno Corp. In side-by-side experiments conducted with polio-seeded water collected from the 30.48 m well, the K-27-pleated filter series consistently yielded higher recovery efficiencies (data not shown).

In an attempt to further eliminate the chance of cross-contamination between samples, reconcentration processes were carried out in three separate laboratories (Lab A - septage; Lab B - 1.52 to 10.67 m wells; Lab C - 15.24 m to 60.35 m, and Northeast wells).

Additional Measurements

Total and fecal coliform enumerations involved the use of the three-tube most probable number method performed in accordance with Standard Methods(19). Ambient sample pH was measured with a portable pH meter (Hach Model 1975). Sample conductivities were measured with a portable SCT Meter (YSI Corp., Model 33).

RESULTS AND DISCUSSION

The constant input of human viruses from the selected multi-family dwelling into a central shallow (i.e. no appreciable depth to groundwater) disposal system presented an ideal situation for studying virus transport to and through the aquifer. The frequency of virus recovery from the central distribution box was 63.6%, with concentrations ranging from 1 to 147 PFU per liter (data not shown). Since many septage samples were highly toxic to cell culture systems (even at 100-fold dilutions), these figures likely represented a conservative estimate of frequency and respective virus concentration. The entrainment of indigenous enteroviruses through the Upper Glacial aquifer was extensive (Figures 2 through 6). Analysis of 60 samples collected from wells located 1.52 to 45.72 m down-gradient from the septage source indicated an overall virus recovery frequency of 30% (18 samples), with concentrations ranging from 1 to 535 PFU per 380 ℓ. A majority of these isolations (14) were made from wells located ≥22.86 m from the waste disposal system. Isolates were recovered from three of the 30.48 m well samples, while the farthest confirmed migration was recorded at the 45.72 m distance. This latter well was sampled on only three occasions then abandoned in favor of a well located 15 m farther down-gradient (60.35 m). Presently, the Laboratory is attempting to confirm plaque-like isolates recovered from this farthest well.

A summary of all data completed to date is presented in Table 1. The frequency of virus isolation was found to decrease with increasing distance from the septage source. This negative correlation ($p < 0.05$, $r = -0.7109$) was confirmed by statistical analysis. The data were interpreted to indicate that viruses were removed (i.e. adsorbed) and/or inactivated during extensive transport.

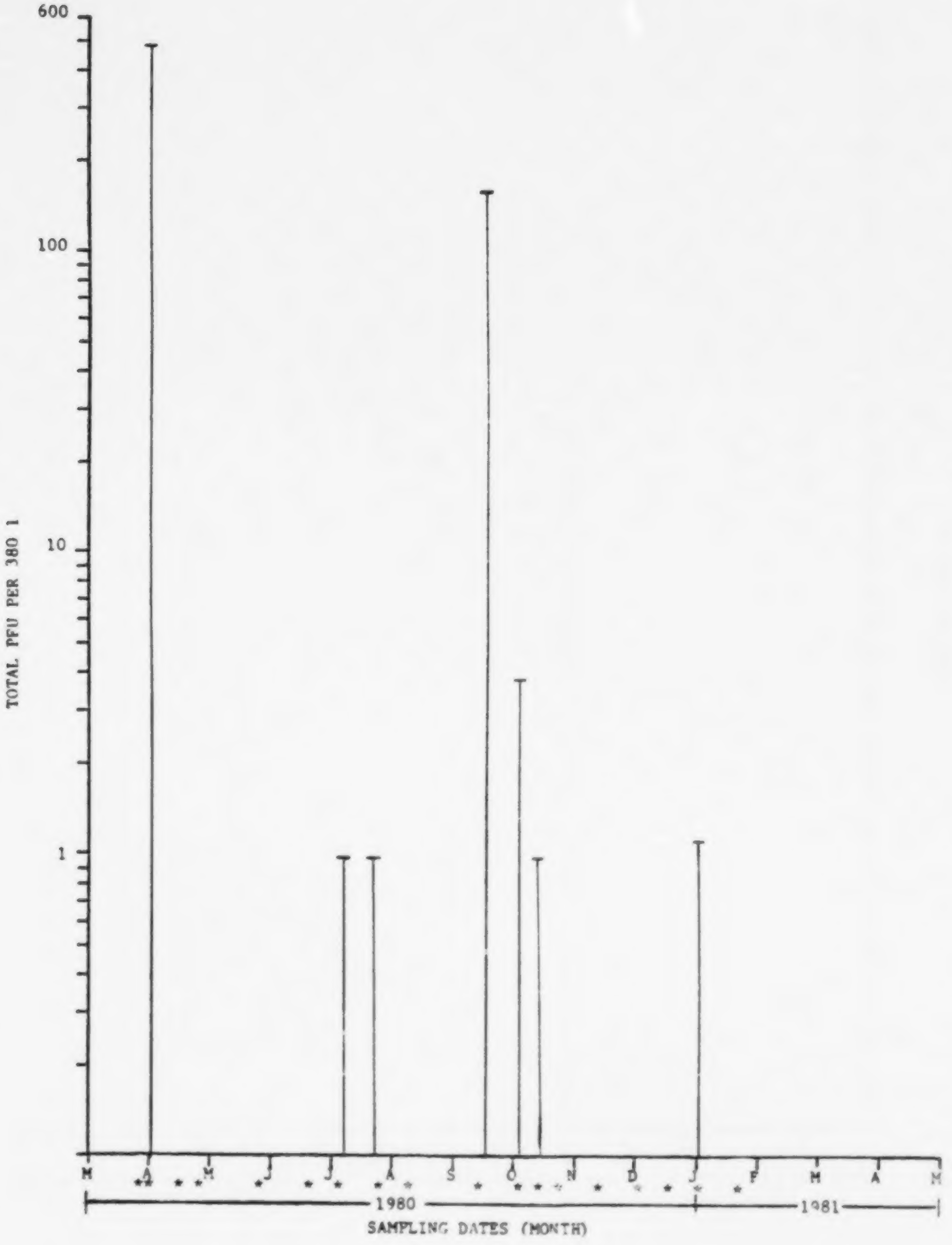

Figure 2. Virus Occurrence in Groundwaters at the 1.52 m Lateral Distance.

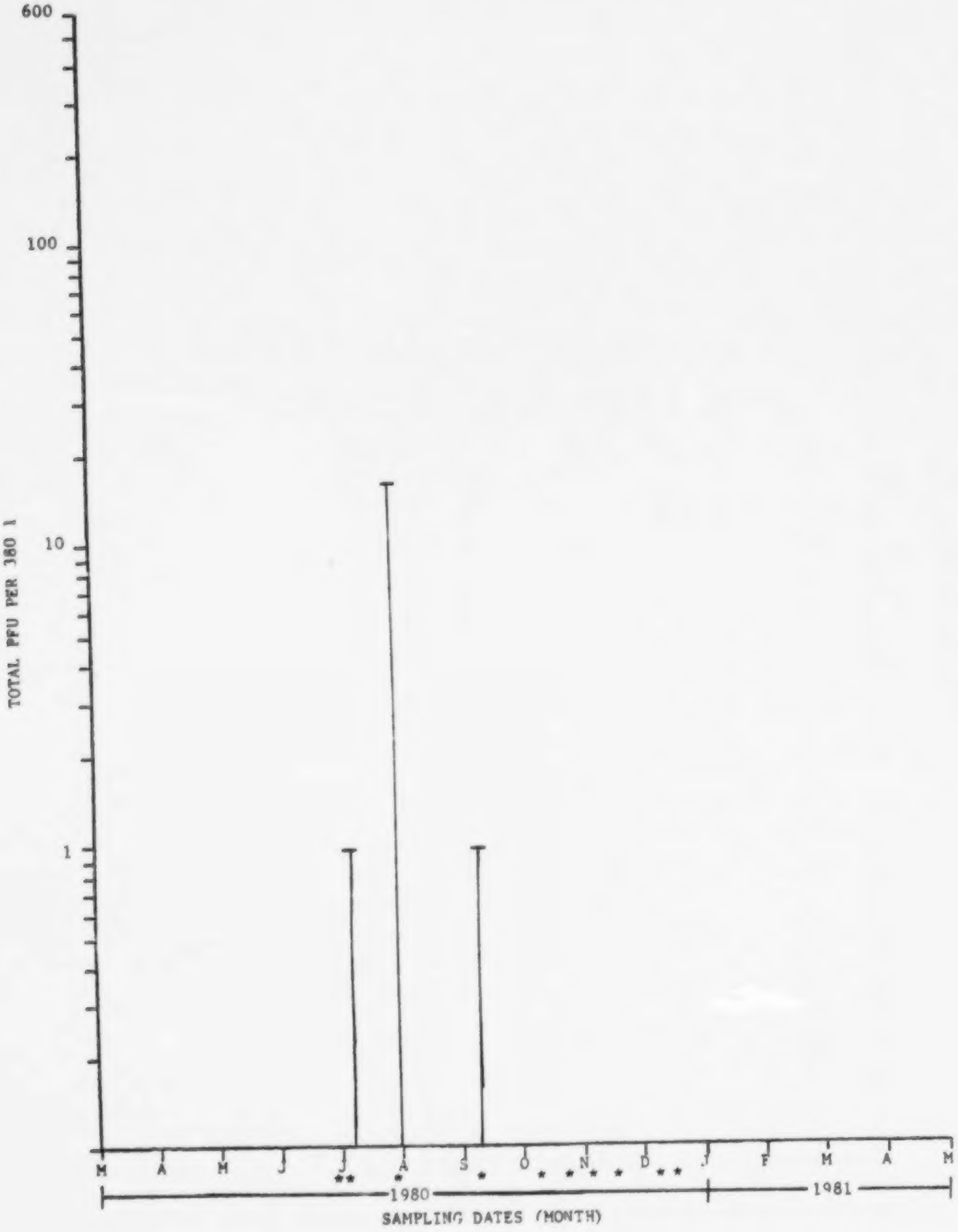

Figure 3. Virus Occurrence in Groundwaters at the 10.67m Lateral Distance.

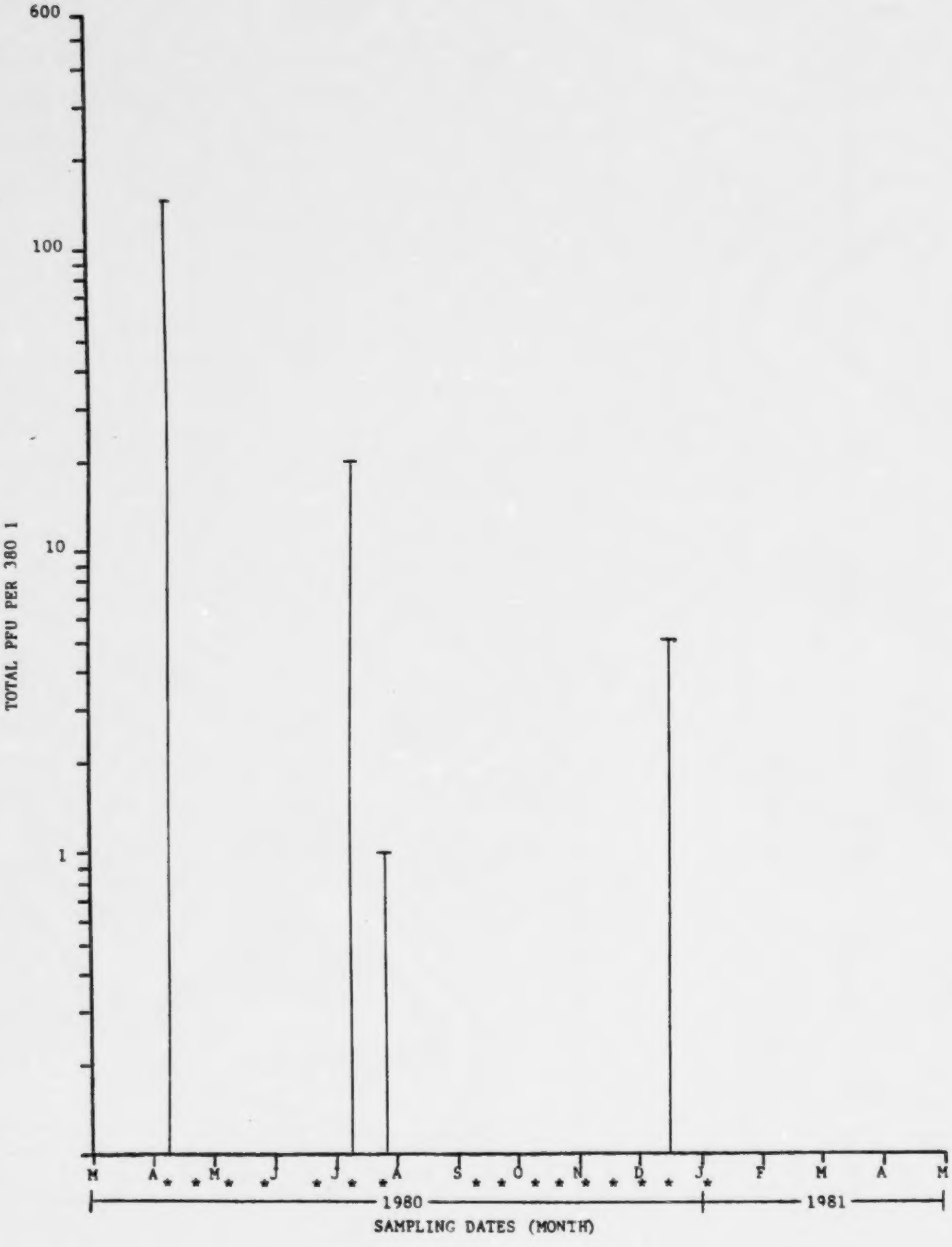

Figure 4. Virus Occurrence in Groundwaters 22.86m Lateral Distance.

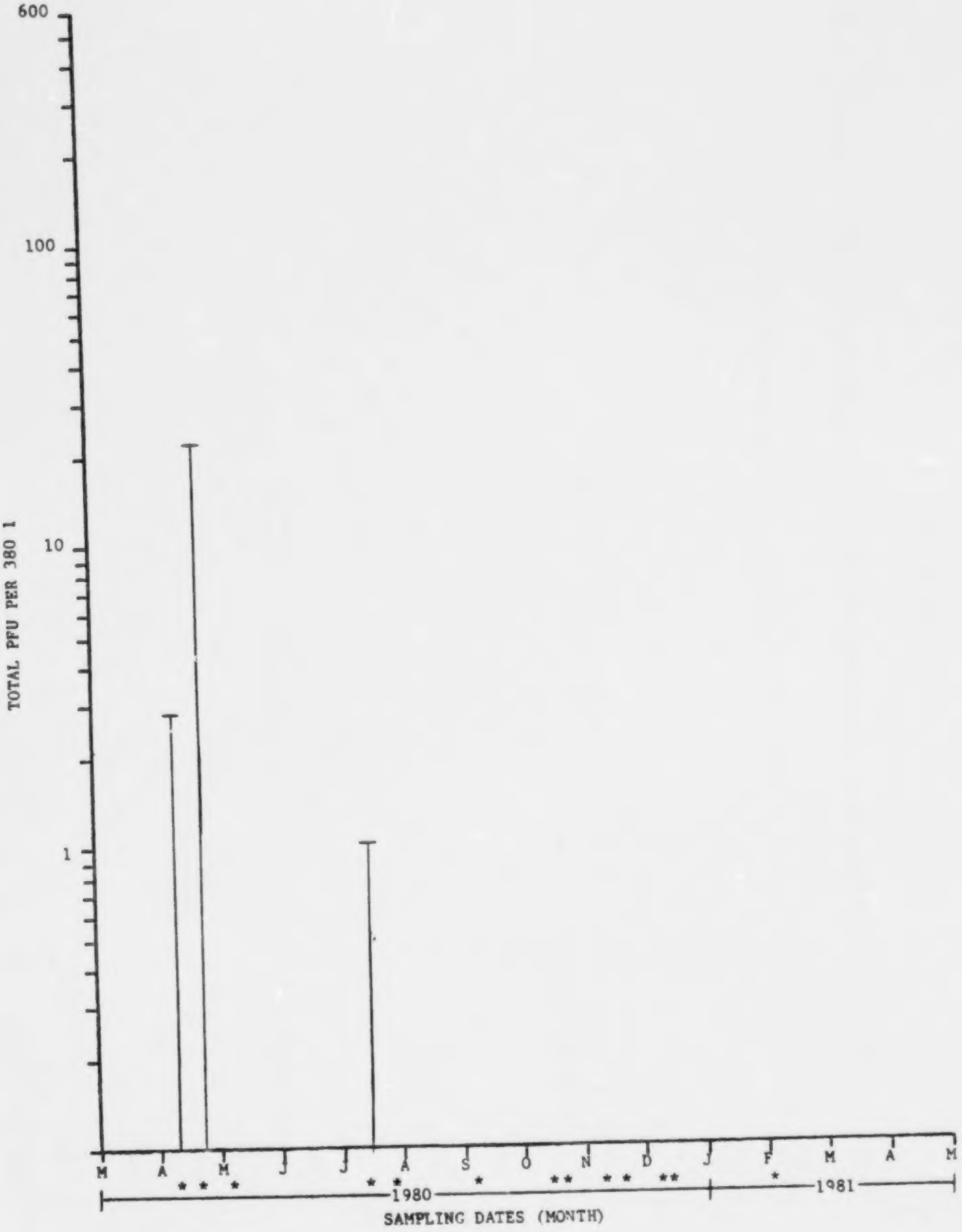

Figure 5. Virus Occurrence in Groundwaters 30.48m Lateral Distance.

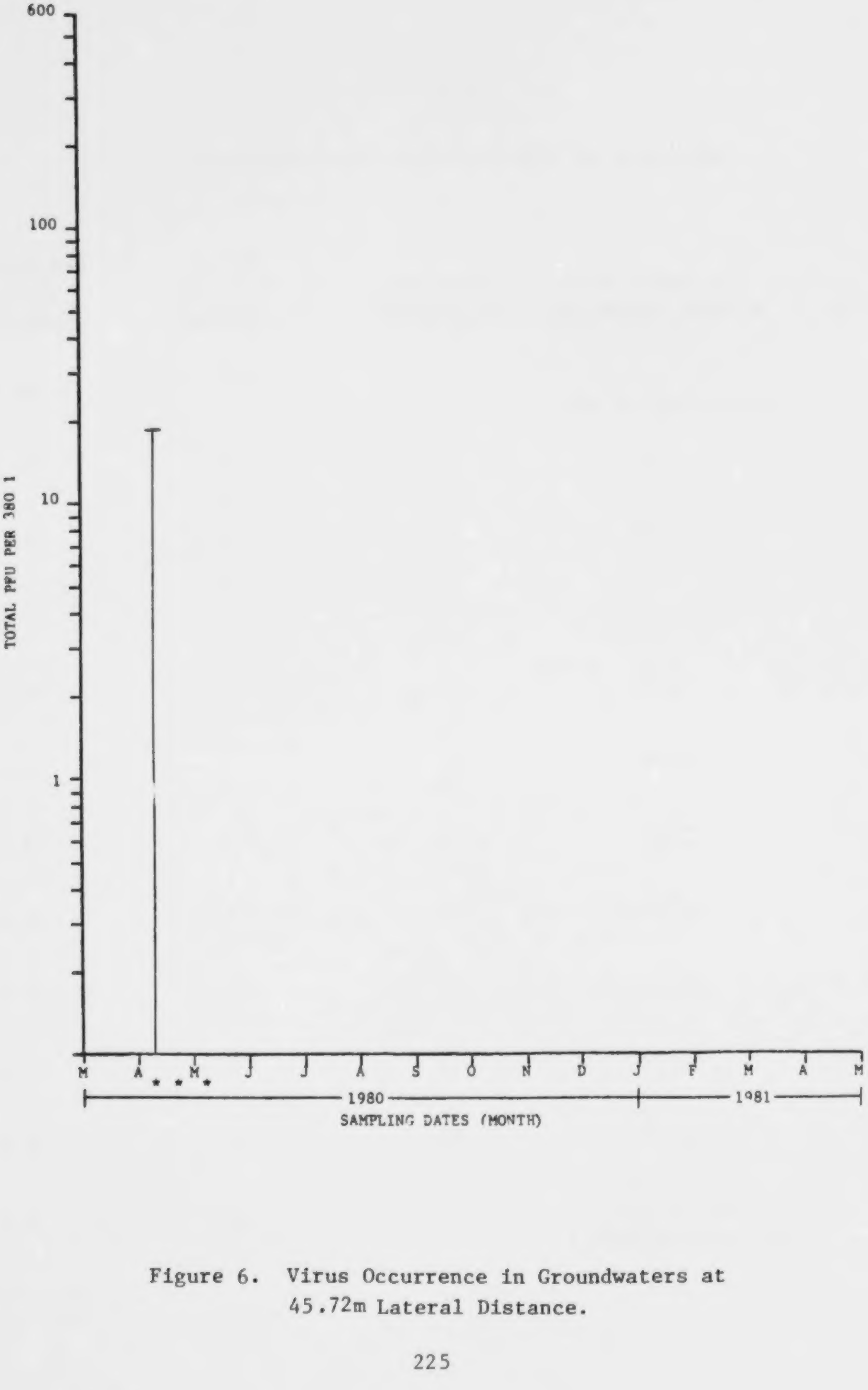

Figure 6. Virus Occurrence in Groundwaters at 45.72m Lateral Distance.

TABLE 1

FREQUENCY OF VIRUS RECOVERY IN GROUNDWATERS

Well No.	Distance From Septage Source (m)	Total No. of Samples	No. of Positive Samples	Frequency of Virus Recovery (%)
1	0 (Distribution Box)	11	7	63.6
2	1.52	18	7	38.8
3	3.05	11	4	36.3
4	4.57	3	1	33.3
5	6.10	11	4	36.3
6	10.67	10	3	30.0
7	15.24	16	1	6.2
8	22.86	16	4	25.0
9	30.48	13	3	23.0
10	45.72	3	1	33.0
11	60.35	11	0	0.0
12	Control Well 34.6 m (Northeast)	6	0	0.0

Seasonal variations were noted in virus recoveries (Figures 2 through 6). Sixteen samples yielded recoveries between April and October, with only two positive samples obtained during the winter months (1.52 m - Jan.; 22.86 m - Dec.). Statistical analyses of virus occurrence versus water quality measurements (pH, conductivity, fecal coliform, total coliform) indicated that no significant correlations existed between the presence of viruses and any of these factors (Table 2). All virus recoveries were made from groundwater which met existing drinking water quality standards as outlined by APHA(19). Fecal coliforms were detected at low concentrations on only two occasions (9 and 4 per 100 ml respectively). These results provide further evidence for the inadequacy of bacterial standards for the assessment of overall biological water quality.

At present, a second phase of groundwater analysis has been initiated. The above data have clearly demonstrated virus transport through the upper levels (1.8 m) of the aquifer. Since screening at this depth gives no indication of vertical virus movement under saturated conditions, and as most of the drinking water wells in the region are screened at much greater depths (6.1 to 12.2 m), a series of deeper screened wells was drilled at the 1.52 and 30.48 m lateral distances. The new well at the 1.52 m distance was screened at 6.1 m, while three wells drilled at the 30.48 m distance were screened at 6.1, 12.2 and 18.3 m, respectively. Sampling at these wells was started in February 1981 and will continue through April.

The implications of lateral transport of viruses through a sole source aquifer which is used for both waste discharge and potable water supply are widespread. In the region under study (Suffolk County), over 750,000 persons use subsurface disposal systems for waste discharge. Simultaneously, over one-half of them rely on the local aquifer as a source of untreated drinking water(20). Many of these inhabitants are housed in densely-clustered housing developments where disposal units are located at minimal distances (30.48 m) from drinking water supply wells. The results of this study illustrate that lateral movement of human viruses from a septage source may be extensive in sandy soils and may eventually lead to contamination of local drinking water supplies. Further study of individual disposal and supply systems in shallow aquifers is required in order to assess the extent of the risk involved. Although the epidemiological significance of the particular viruses under study is debatable, it is clear that they represent an alternative system for indicating fecal contamination of groundwater which may prove to be more accurate than existing bacterial standards.

TABLE 2

PRODUCT MOMENT CORRELATION MATRIX DEFINING THE RELATIONSHIP BETWEEN TOTAL PFU AND FOUR INDEPENDENT VARIABLES

	Correlation Coefficients			
Occurrence of Virus in Groundwaters at	Total Coliforms per 100 ml	Fecal Coliforms per 100 ml	pH	Conductivity
1.52 m	-0.0763	-0.1020	-0.2585	-0.2193
10.67 m	-0.1276	-0.0597	0.4881	-[d]
15.24 m	0.0951	-[b]	0.0959	-0.0280
22.86 m	-0.0788	-0.0788	-0.1251	0.2259
30.48 m	-0.0760	-[b]	0.0162	0.4371
45.72 m	0.5000	-[b]	-[c]	0.9007
60.35 m[a]	-	-	-	-

a No PFU isolated from well
b No fecal coliforms in wells
c No variance in pH readings
d No conductivity readings available

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TRANSPORT AND FATE - VIRUSES IN GROUNDWATER: JAMES VAUGHN

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: Salvato

You indicated that there were some 20+ leaching pits. Would you happen to know the actual depth of the leaching pits and the depth at which the ground water is impacted to give you some indication of the unsaturated zone?

A: Vaughn

This is in the paper. There was a 3 meter unsaturated zone between the basic leaching rule and the static ground water level. Of course, the static level may vary as much as 16 feet under drought conditions. During the time of the study, there were approximately 3 meters of unsaturated zone. The overall depth to the ground water was approximately 25 feet in the study area.

Q: Gregg

I appreciate the problems you may have had in doing this study, in terms of informing the public or otherwise, but I wonder if you had any opportunity to put into the water system of the housing units fluorescein or any tagged materials so that you could find out how quickly there was flow from sewage effluent to the area in question?

A: Vaughn

We had originally planned to do some studies with a guanidine-resistant strain of poliovirus which we developed at Brookhaven, in order to get some idea of how fast the material moved out.

However, the economics became unwieldy. We had collected some 275 samples over that year, most of them at least 100 gallons in volume and the whole project cost was $90,000. We simply weren't able to add a tracer experiment.

Movement out of the septic system is probably quite rapid. We talked yesterday about retention times of 2 to 5 days. It's probably in that ball park. The septic tank itself was obviously underused, as it had been designed for much larger septage loads. In treating one of the distribution pools, we found virus 60 percent of the time. We would have found more, I think, but many times the material was just too toxic to our tissue culture cells. While testing that particular distribution pool, we were not necessarily testing fresh material. We would have liked to have known how fast it moved out of that system. It seemed to be quite rapid. Viable viruses were definitely coming out.

Q: Rohlich

You mentioned that most of the isolations occurred in the spring to fall. Do you see any possibility of a relationship to precipitation and the fact that most of the isolations occurred during this time? In other words, could there have beem more hydraulic movement as the result of precipitation occurring?

A: Vaughn

We considered this. In fact, during a sampling day the weather conditions were recorded. We had a very dry spring last year, a very wet summer, and not much rain during the fall. I really haven't looked at the correlation between precipitation and overall virus occurrence, although we can do this. It may be interesting to try it.

Q: Lennette

Actually there were listed some viruses that were unidentifiable. This is a matter of semantics. Did you try to identify them and couldn't, or did you just not try to identify them?

A: Vaughn

From this study?

Q: Audience

Yes.

A: Vaughn

For this particular study we only attempted to identify a few of the viruses, again because of expense limitations. We used the LBM system and anything that we couldn't identify by that we called unidentifiable. We have identified members of the polio, the coxsackie and the echo group. Based on the types of plaques we got, the time it took for the plaques to develop on these particular cells, it would appear that we were getting a lot of coxsackie. This is consistent with some of our laboratory data which indicated that coxsackie readily moved through soil. I believe Mark alluded to this yesterday. Some earlier work by Chuck Gerba indicated that they were finding coxsackie in ground water after the precolation of effluent through soil. Our initial isolations in the 208 study a couple of years back show larger numbers coxsackie and echo viruses getting into ground water.

These particular virus types seem to be better able to move through soil.

Q: Gregg

This may be a ridiculous question, but in terms of just the flow dynamics from the main tank where the sewage goes, has anyone looked at the sewage in the main tank to see whether the virus particles remain suspended at an elevation, or whether they tend to settle down in particulate matter so that you have a gradient of concentration, conceivably in the main holding pool? Has anyone ever looked at this?

A: Vaughn

Well, we have not looked at it here, but I think there was a diagram yesterday that indicated that viruses would go both ways. In other words, they will settle. That would be a lot of particulate associated viruses that will be coming out in the sludge and there are those that will even be associated with small particles or unassociated leachates (unassociated viruses in a septic tank is hard to conceive). They are usually going to be attached to something, but they may be attached to something that is small enough to float. So the answer to your question is probably they will be in both places, but I don't know if there is a gradient. There are some recent studies on the form the viruses are in when they come out of sewage treatment plants.

Q: Salvato

What was the effective size and uniformity coefficient of the sand? Do you have any idea?

A: Vaughn

The soil in that area is coarse sand and gravel. It's very coarse sand, and it's compacted. I believe it is of the C or B horizon type. It is extremely porous all over the island.

Q: Salvato

So the discharge of the sewage was really rapid through the leaching pits in contrast to what it might have been if the sewage was distributed in the lateral trench, for example, where you have a lower rate of application.

A: Vaughn

It may have been, depending on how close it was to the agricultural trench which was set. We have done a lot of studies on infiltration rates, not at this but at another site that uses rapid infiltration, and if you scarify the soil in a recharge basin there, you can realize a 150 cm per hour infiltration rate for a very long period of time. The soil there, if I can refer to a paper by Gerba where they describe a more or less ideal type of virus adsorbing soil, I would have to say that the Long Island soil is the antithesis to that. There is no reason why viruses should absorb to that soil, although they do. There is little clay, less than 1 percent. There is very little organic material.

Q: Salvato

I just want to point out the fact that Long Island sands are very clean, coarse sands which would permit travel of pollution long distances. Not only viruses but perhaps other microorganisms.

Q: Rycheck

As I understand your virus samples were 100 gallons and your samples for coliforms 100 ml. Would you spend a moment on Table 2? I don't quite see the relationship of Table 2 with what you actually did.

Q: Rycheck

Is the conclusion from Table 2 that there is no correlation?

A: Vaughn

It shows the correlation between virus occurrence at these wells. There appeared to be no correlation except at the 45.72 m well. It shows no relationship between occurrence of viruses and pH, or conductivity. Based on the statistical analysis there appeared to be no correlation, so I was told, except for this one point in the 45.72 m. We only took three samples from that well so it is statistically unwise to try to make the point that there was any correlation. We found in essence no correlation between virus occurrence and any of these other particular water quality factors.

Q: Audience

What is the implication?

A: Vaughn

I think the obvious one is that one cannot rely on (in this particular ground water situation) these particular factors to determine whether or not viruses are present. In other words, the way swimming pools are judged to be O.K. is by chlorine content, or sewage effluents are judged to be biologically acceptable based upon chlorine residuals. One could not look at any of these factors and say, for example, that conductivity is high so there must be viruses, or conductivity is low, therefore there are no viruses or total or fecal coliforms. By the way, all water quality measurements were well within the current standards for drinking water.

Q: Davidson

From the summary table it is hard to know what the original data looked like but, if I understand your results correctly you are saying that if you found a single plaque forming unit (pfu) per hundred gallons of water and the coliform test was negative then a lack of correlation existed.

A: Vaughn

Well, this was freqneucy of occurrence throughout the study, not just individual occurrence, the total number of viral occurrences versus those of coliforms.

Q: Davidson

What I am getting at is what is the difference in magnitude between the two tests? What importance should we place in the fact that you may find a single plaque forming unit in 100 gallons of water and yet the coliform test may be negative? Is this an important finding?

A: Vaughn

That would be for the standard makers to determine. I don't know and in front of this body it would be tempting fate to say that it means something. There has been a standard proposed for drinking water.

Q: Lennette

Let me correct the record here. This often crops up as a proposed standard and as a newcomer and a novice to the field I began to read the literature and I saw a whole string of references pertaining to this standard which went back several years. So you would think there was a great consensus that this is a reasonable standard. I traced these back from, say, Reference 27, 26, 25 and each guy was quoting all of his predecessors. Finally I got back to Joe Melnick and Joe had apparently just made a casual, off the cuff, suggestion which has since been engraved in stone.

Q: Davidson

How much did the pH change during the season? How much did the conductivity change during the season?

A: Vaughn

There were a few bursts in conductivity. The pH remained pretty much the same, slightly acidic between 5 and 5.5 except in the drinking water well. As I was telling someone the other day, water softeners are put in the drinking water. Sometimes the pH gets up to around 9.5 or 10. We rarely saw big pHfluctuations in the observation wells. We did on occasion see bursts of conductivity at the near wells, those closest to the distribution boxes. We also had problems with iron oxide on three or four occasions which caused clogging problems with the filters. I can't tell you exectly what the fluctuations were.

Q: Davidson

I'm not surprised that you obtained a good correlation between pfu and pH. If pH does not change over more than 0.5 one would expect a good correlation. The same thing with conductivity. Statisticians are excellent colleagues, but you need to use some caution before turning numbers over to them to develop correlation coefficients. Computers will also do an excellent job but interpretation is far more important.

EPIDEMIOLOGICAL APPROACHES

VIRUS OCCURRENCE IN GROUND WATER

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ABSTRACT

Most studies on the occurrences of viruses in ground water can be divided into two groups: 1) isolation of viruses from ground water sources and drinking water; and 2) isolation from sites where experimentation of ground water recharge or land application of wastewater is being practiced. Information on the occurrences of viruses in ground water is still limited because adequate methods for the concentration and detection of human enteric viruses in large volumes of water have been available for only the last few years. This paper reviews viruses isolated from ground water associated with waterborne disease outbreaks, isolation of viruses from drinking water wells, and isolation of viruses from beneath land application sites and in the vicinity of septic tank systems. The current body of information on the occurrences of viruses in ground water is too limited to be used for the development of guidelines on estimating safe distances between waste sources and drinking water wells. Field studies are sorely needed to define such guidelines.

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VIRUS OCCURRENCE IN GROUNDWATER

Introduction

Only in the last decade have adequate methods become available for the concentration and detection of human enteric viruses in large volumes of water. Such methods were believed necessary to adequately assess the occurrence of enteric viruses in drinking water and other types of water and wastewater. One of the first applications of this technology was the sampling of groundwaters beneath wastewater land application sites (1, 2). Thus, most of our knowledge on the occurrence of viruses in groundwater rests upon studies conducted only over the past few years.

Most of the studies can be divided into two groups: (i) isolation of viruses from groundwater sources and drinking water and (ii) isolation from sites where experimentation of groundwater recharge or land application of wastewater is being practiced. The differences are ones of convenience and the prior knowledge of the source and type of pollution. It is obvious that in the first group, the presence of viruses in water is more difficult to demonstrate unless the source has been monitored closely because the contamination is most often unexpected. Usually, samples are taken only after an outbreak of overt illness or some other event has taken place, so that the contamination is often no longer present.

Viruses in Groundwater Associated with Waterborne Disease Outbreaks (Table 2)

The prime concern of the presence of viruses in groundwater is the transmission of infectious disease. Of the 550 documented waterborne disease outbreaks which occurred from 1946 through 1977 almost 65% have a probable viral etiology (hepatitis A, poliomyelitis, gastroenteritis). Because of previous limitations in concentration methodology and the sudden occurrence of outbreaks few reports exist on the isolation of viruses from waters associated with waterborne disease outbreaks. In addition, no methodology currently exists for the detection of the Norwalk agent, probably responsible for many of the gastroenteritis outbreaks. Although the Norwalk agent cannot be isolated from groundwater, it certainly occurs as evidenced by the growing list of groundwater disease outbreaks associated with this virus.

There are currently eight groundwater disease outbreaks known to be associated with the Norwalk agent, as based on serological evidence and the isolation of the virus from stools of infected individuals (Table 1).

TABLE 1

WATERBORNE GASTROENTERITIS OUTBREAKS ASSOCIATED WITH NORWALK-LIKE AGENT(S)*

Location.	Year	Water source	Number of cases
Colorado	1976	well	418
Pennsylvania	1978	well	350
Pennsylvania	1978	well	120
Washington	1978	well	467
California	1979	groundwater	30
North Carolina	1979	well	146
Pennsylvania	1979	well	151
Maryland	1980	well	139

*Modified from (3)

TABLE 2

ISOLATION OF VIRUSES FROM DRINKING WATER WELLS ASSOCIATED WITH WATERBORNE DISEASE OUTBREAKS

Location	Virus Types	Concentration Method	Reference
Florida	Echo 22/23	Membrane Adsorption	5
Michigan	Polio 2	PE60/centrifugation	4
Texas	Coxsackie B3, B2 Hepatitis A	Membrane Adsorption	7
Maryland	Polio 1 Echo 27, 29 *U	Membrane Adsorption	8
Israel	Polio 1; U	Organic Flocculation	6

*U = unidentified

Mack et. al. (4) were the first to isolate a virus from groundwater associated with waterborne disease illness. Five-gallon samples were taken from the 30.5-m-deep well and were concentrated by a PE60/centrifugation technique. Coliform levels in the well ranged 0-16/100ml, but no Salmonella or Shigella were found. The source was a waste drain field that allowed sewage to enter the well by passing through 5.5m of clay, 2.5m of shale, and 22.5m of limestone. Although poliovirus was isolated from the well, it was not suggested as the causative agent of the gastroenteritis outbreak. That outbreak was probably attributable to some other virus (such as rotavirus or the Norwalk agent) which could not be detected by the methods available at that time. Also, it is evident that soil type, structure, and proximity of a well to a source of sewage play an important role in the contamination of groundwater sources, as the virus in this case penetrated 20.5m through several soil profiles, and traveled 91.5m laterally.

Wellings et. al. (5) reported the isolation of an echovirus 22/23 complex in 100-gal samples from a 12.2-m-deep well during an outbreak of gastrointestinal illness at a migrant labor camp in Florida. The well was located 30.5m from a solid waste field, and was in the middle of an area bordered by septic tanks. The echovirus was isolated from sewage, from potable well water containing 0.4-0.6mg/L residual chlorine, and from stools collected from individuals living in the camp. Furthermore, it is interesting to note the occurrence of 15 cases of hepatitis A in the camp some 6 weeks later.

Thus, the virus was probably present because of well water contamination from the septic tanks, and existed in chlorinated water in the absence of evidence of bacterial contamination. It was surmised that the chlorine was ineffective against the virus which was probably associated with solids introduced with the sewage. Even though this level of treatment reduced bacterial counts to undetectable levels, the virus survived. This raises concern about the efficacy of chlorine disinfection of contaminated groundwater, based on currently accepted standards.

Shuval (6) reported the isolation of poliovirus from a well under suspicion during an outbreak of hepatitis A in Israel. Poliovirus type 1 (4.0 7.2 PFU/L) was recovered from a single well, as were coliform bacteria.

In June 1980, an outbreak of gasterointestinal illness and infectious hepatitis was found to be associated with drinking water wells in the city of Georgetown, TX. The attack rate of gastroenteritis among the 10,000 individuals living in the areas supplied by the wells was almost 80%. An increased number of cases of hepatitis A began to appear early in July 1980. Georgetown received its drinking water from wells with no water treatment other than chlorination. At the time of the peak of the gastroenteritis outbreak, 400-1100 liter samples of well water and chlorinated drinking water were taken for virological analysis. Some well samples were found to be heavily contaminated with coliform bacteria before receiving chlorination. Potable water samples taken for virological analysis contained 0.8mg/L residual chlorine. Coxsackievirus B2 and B3 were isolated from the well water. Coxsackievirus B3 was also isolated from a chlorinated tapwater sample. Hepatitis A antigen was identified by radioimmunoassay in one of the well water samples. This is the first demonstration of hepatitis A in water used as a source for potable water prior to a suspected waterborne disease outbreak.

Also, in 1980, enteric viruses were isolated from iodinated groundwater at a summer camp in western Maryland during an outbreak of gastroenteritis (8). The water supply for the camp was a 95-feet well which, during the outbreak, contained 0.7-1.0 ppm iodine (recommended 0.3-0.5 ppm). The coliform count in the raw well water was 5000/100 ml but all drinking water was within allowable standards, during and after the epidemic. Preliminary evidence has indicated that the Norwalk agent may have been responsible for the outbreak. To date, Poliovirus 1 and echovirus 27 and 29 have been isolated from the well water.

Isolation of Viruses from Drinking Water Wells

Unfortunately, few systematic studies on the occurrence of viruses in groundwater over a region have been conducted. The largest to date was done by Marzouk et. al. (9) who collected 20-440-L samples of groundwater from 3-m-deep wells in Israel.

Twenty of 99 samples were found to be positive for viruses. The viruses isolated included coxsackievirus B6, echoviruses 6 and 7, poliovirus 1, and several unidentified types. Total bacteria, fecal coliform, and fecal streptococal concentrations ranged from $0-10^4$, 0-200, and 0-100, respectively, per 100 m . In at least one case, viruses were detected in samples that contained no total bacteria, while 12 of 17 samples that were negative for fecal coliforms and fecal streptococcus were positive for viruses. No statistical correlation could be demonstrated between indicator bacteria and the presence of viruses, which suggests the failure of bacterial indicators to represent viral contamination adequately.

Morris (10) isolated poliovirus type 2 in two samples of groundwater in England. Concentrations of 1 plaque-forming unit (PFU)/40 L and 4 PFU/10L were detected in these 5-100-L samples.

Walter and Rudiger (11) found that eight of 62 10-L samples from a groundwater source for a large water supply system contained viruses. Echoviruses 3, 6, and 30; coxsackieviruses B1, B4, and B5; and several unidentified viruses were recovered. No coliform data were given in the report.

TABLE 3

ISOLATION OF VIRUSES FROM DRINKING WATER WELLS*

Location	Virus Types	Concentration Method	Reference
Germany	Echo 3, , 30; Coxsackie B1, 4; U	$Al\ (SO_4)_3$	11
Israel	Echo 6, 7; Coxsackie B6; Polio 1; U	Filter Adsorption	9
England	Polio 2	$AlCl_3$l Filter Adsorption	10
Ghana	Polio 1; Coxsackie B3	Centrifugation	13
Mexico	Rota; Coxsackie B4, B6	Membrane Adsorption	33
India	U	Iron Oxide	12

*Unidentified

Rao et. al. (12) detected viruses in six different wells in Nagpur, India. Concentrations of 2-8 PFU were recovered from 40-L samples collected at depths of 12-24m. No attempt was made to identify the isolates.

Addy and Otatume (13) isolated poliovirus 1 and coxsackievirus B3 from three of eight wells in the greater Accra region in Ghana. Those wells were dug specifically to provide water for human consumption. Also, a report from China (20) indicates that waterborne hepatitis traceable to well water contamination has caused several large epidemics

These studies illustrate the need for monitoring and maintaining the integrity of groundwater sources. It is obvious that, in many cases, groundwater assumed to be safe is not.

Land Application Sites
(Table 4)

Even though there have been no reports of disease outbreaks associated with land treatment of sewage wastes, there are a growing number of studies concerning the detection of viruses in groundwater after wastewater application to land or direct groundwater recharge.

Wellings et. al.(14) demonstrated vertical and lateral movement of virus in secondary effluent discharged into a cyprus dome (a wetland ecosystem). Poliovirus 1, coxsackievirus B4, and echoviruses 7, 11, and 14 were recovered from 3-m-deep wells in three of 71 samples, at concentrations ranging 4-353 PFU. Viruses migrated 7-38m laterally from the application point and survived at least 28 days.

The soil at this site ranged from a top 0.6-m layer of black organic soil (4-12% clay) to a sandy clay and a solid blue clay with a permeability of 3×10^{-2} cm/min to 3×10^{-6} cm/min. Thus, the viruses moved horizontally as well as vertically and survived many days under ambient conditions.

Wellings et.al. (1) also recovered viruses from groundwater after spray irrigation of secondary sewage effluent onto an Imolokee sand (little or no silt or clay). Of particular interest in this study was that viruses survived chlorination, sunlight, spraying, and percolation through 3-6m of sandy soil; furthermore, after a period of heavy rains, a burst of viruses was detected in samples that had previously been negative. These studies demonstrate that soil type, rainfall, and other factors can affect viral movement into groundwater, and that viruses are capable of surviving long periods which, when combined with the ability to move long distances laterally, could lead to wide dispersal through an aquifer.

TABLE 4

ISOLATION OF VIRUSES BENEATH LAND TREATMENT SITES*

Site location	Type of site	Virus types	Maximum distance of virus migration (m) Depth	Horizontal	Reference
St. Petersburg, FL	S	Polio 1; coxsackie B4; echo 7	6	—	1
Gainesville, FL	S	Coxsackie B4; Polio 1, 2	3	7	14
East Meadow, NY	R	Echo 12; U	11.4	3	15, 16
Holbrook, NY	R	Echo 6, 21, 24, 25; U	6.1	45.7	15, 16
Sayville, NY	R	U	2.4	3	15, 16
12 Pines, NY	R	Polio 2; U	6.4	—	16
North Masapequa, NY	R	Echo 11, 23; Coxsackie A16	9.1	—	15
Babylon, NY	R	Coxsackie B3; U	22.8	408	15
Ft. Devens, Mass.	R	U	28.9	183	18
Vineland, NJ	R	Polio; coxsackie B3; echo	16.8	250	19
Phoenix, AZ	R	Coxsackie B3	18.3	3	22
Lubbock, TX	S	Coxsackie B3, A9 Echo 12	30.5	—	20
Kerrville, TX	S	U	1.4	—	23
Dan Region, Israel	R	Polio 1, 2, 3	31-67	60-270	24
England	S	Polio 2, 3 Coxsackie B4, 5	19.4	—	25

*R = rapid infiltration
S = slow rate infiltration
U = unidentified

In a series of reports enteroviruses were found near four groundwater recharge sites, a stormwater recharge basin, and from groundwater under a sanitary landfill in New York (15-17). These sites have soils of coarse sand, fine gravel, and 1-2% silt. At the groundwater recharge sites, viruses were recovered at depths up to 11.4m and at distances up to 45.7m from the injection point of secondary or tertiary chlorinated effluent. As much as 22-33% of the 100-gal samples at the four sites were positive for viruses, with concentrations of 1.3-10.6 PFU/gal. In addition, total coliforms and fecal coliforms were found in these samples. In order to reach the groundwater, viruses moved through 5.5-9m of the overlying soil.

Both the landfill and stormwater recharge basin also yielded viruses. At the landfill site, viruses were detected at depths of 22.8m and up to 408m downstream. Coxsackievirus B3 and other unidentified viruses were detected. At the stormwater recharge site, samples taken at 9-m depths directly in the basin were positive for echoviruses 11 and 23 and for coxsackievirus A6. This contamination may have originated from runoff from cesspools in the area.

Schaub and Sorber (18) reported on a study of viruses in groundwater under rapid infiltration cells at Ft. Devens, Mass. The soil consisted of silty sand and gravel underlain by bedrock. The groundwater contained viruses at depths of 29m and lateral distances of 183m, with concentrations of about 8.3% of the applied effluent.

At the Vineland, N.J., rapid infiltration site (19) primary effluent was applied to Cohansey sand and coarse gravel. Viruses were found at 16.8-m depths and up to 250-m lateral distances in 19 of 40 samples. Polio-, echo-, and coxsackieviruses were identified. Total coliforms and fecal coliforms were found consistently at depths up to 6m beneath the recharge basins. Total coliforms also occasionally occurred at greater depths and downstream. In contrast, no fecal coliforms were found in samples taken below 9.1m and coliforms occurred only once in a shallow well 50m down-gradient. Thus, viruses penetrated deeper into the ground and moved longer distances than did the coliforms.

At two land treatment sites where sewage is used to irrigate cropland, both positive and negative virus isolations have been made (20, 21). At the Lubbock, Tex., site, coxsackievirus B3 was isolated from a depth of 30.5m; at Roswell, N.M., no virus isolates were detected in samples taken from 3-30-m depths. In the latter case, irrigation is seasonal and intermittent, whereas application at the Lubbock site is continuous.

Septic Tank Systems

Overflow from septic tanks and cesspools has been estimated to be responsible for 42% of the outbreaks, and 71% of the illness caused by using untreated groundwater in nonmunicipal systems (26). Yet, almost no field work has been done on the occurrence of enteric viruses in groundwater near septic tank systems. Recent studies using "seeded" marker viruses added to septic tanks indicate that viruses can travel significant distances in the subsurface from these sources (27, 28). Two recent studies currently underway have reported the isolation of enteric viruses from wells near septic tank systems (Table 5) (29, 30).

In the study in Texas, (Table 5) an enteric virus was isolated from an abandoned well 25m distance from a septic tank system serving a mobile home park. No viruses were isolated from another well 300m distance from the same drainfield (29). In a survey of septic tank systems on Long Island, N.Y. enteric viruses have been detected in sampling wells as far as 9.4m from a known septic tank source and at depths as great as 45.7m (30).

Summary and Recommendations

As is apparent from this review, current information on viruses in groundwater is limited. Our knowledge in certain areas is especially deficient. Despite the importance of septic tanks as a potential source of viruses in groundwater, almost no studies have been done on this source. Technology is currently available for the concentration and detection of enteroviruses in large volumes of water. This technology should be applied to virus surveys of groundwater. Such surveys should focus on rural private sources and small communities where groundwater is often used with minimal or no treatment.

Results of previous surveys on the bacteriological quality of groundwater indicate that viral occurrence is more widespread than might be believed. Furthermore, many of the previous systems used for virus concentration had efficiencies of 2-3%, and were limited to the detection of enteroviruses (31). Now, however, systems with efficiencies of 30% and greater are available (32). It is evident that virus contamination of groundwater does occur when human wastes are applied to the land and can travel long distances in groundwater under the proper conditions.

The current body of information on the occurrence of viruses in groundwater is too limited to develop guidelines on safe distances between waste sources and drinking water wells. Field studies are sorely needed to define such guidelines. The last decade has seen major developments in technology for virus detection in water, it would appear time to apply this technology to answer some of the major questions concerning viral contamination of groundwater.

TABLE 5

ISOLATION OF VIRUSES FROM WELLS NEAR SEPTIC TANKS*

Location	Virus Types	Sampling Well Depth (m)	Distance from Septic Tank (m)	Reference
Texas	U	5	25	29
New York	U	9.4	45.7	30

*U = Unidentified

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VIRUS OCCURRENCE IN GROUND WATER: CHARLES GERBA

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: Morrison

Charles, may I serve as the devil's advocate in making a comment before asking you a question. I think what we have seen happen in chemical toxic agents in the last 12 or 15 years is as each new instrument is developed, enforcement agencies have added another zero after the decimal point on acceptable levels. Is this going to happen in our detection methodology for viruses? In other words, as the virologists become more sophisticated in their laboratory procedures are we going to see more viruses in ground water samples without having any correlating health effects with the isolations?

A: Gerba

Yes, I think you will see that as technology improves. I think we have seen it over the last decade. As technology improves we have been able to isolate where we couldn't previously find viruses in ground water. I think that is one of my concerns. Maybe I should reflect on that and virus standards. Suggestions have only been made on virus standards, but whether these adequately reflect the human health situation is really not known. There is always the pressure I think to have some type of standards or some type of drawable line somewhere on the occurrence. I don't really feel that it is my place to say that the standards should be established or what these standards should be because as you pointed out, when such standards are moved forward they become very difficult to meet and there is a lot of concern about them when they are tolerated. I really don't think I have the answer for your question because I feel that it is in the hands of the decision maker, but that is the concern from my standpoint too.

Q: Morrison

You said it is the responsibility of the decision maker; however, I think the charge that we all feel here is that if the scientist doesn't make the decisions then other non-technical disciplines will make them for us, so I think we are all in the same boat in the sense that we should like to have the best evaluation information going. That's it, I didn't expect an answer.

Q: Sobsey

I can share Dr. Morrison's concern about where improved methodology might be taking us when it comes to looking for viruses in environmental samples. Indeed there is a negative side to that because the methodology is getting better in terms of recovery efficiencies and it is getting easier to do virus isolations from field samples. We can expect to see more data being generated. In a sense, these data are out of context because they are not related to any obvious situations involving disease outbreaks. However, with such improved methodology we are also in a position to be more responsive in terms of investigating outbreaks or looking into situations where we suspect there may be problems related to viral contamination of water. Previously, because of limitations in methodology, we were unable to investigate such situations. The investigation that I recently did on a waterborne gastroenteritis outbreak in Maryland is a good example. I got a telephone call from people at the Maryland State Health Dept. saying that there was a waterborne gastroenteritis epidemic at a summer camp in Maryland, and they suspected that the well water supply was the source of contamination. Their question was: is it possible to look for viruses in the water supply? Not necessarily the viruses responsible for the outbreak but any viruses at all? What we were able to do at relatively low cost was to mail the virus concentrating filters to the state health department people, they processed the water on site, and the filters were returned to my laboratory. Viruses were recovered from the filters and further concentrated and isolated in cell cultures. Indeed it was possible to demonstrate that there were enteric viruses in that well water supply. A few years ago we could have never even confirmed the presence of viruses in the water supply. Now we are in a position to be responsive to an outbreak situation. I also think, and I share the opinion of Dr. Gerba and others, that with this improved methodology we are in a much better position now to examine situations where we suspect there is viral contamination of a ground water supply or some other water source. In the past, the available methodology made it difficult to investigate those situations, but now we can investigate.

A: Gerba

I think that Dr. Sobsey's comment is relative to the Georgetown outbreak in that hepatitis was isolated in the water three weeks before the first cases. If we had the methods at the time we would have been able to determine if the population had indeed been exposed to hepatitis A by the water route before the outbreak occurred. I think pertinent to applications, that this type of technology using rapid rate amino assay systems can tell whether an exposure to the population has happened. I would like to put virus standards into perspective a little bit since the committee may not be familiar that there are recommended standards made by an expert committee of the World Health Organiation some years ago. They recommended guidelines for the minimum concentrations of viruses in drinking water as one virus in one thousand to one hundred liter range. The European Economic Community has also recommended guidelines for the occurrences of viruses in raw water, and treated water sources. There are recommendations that have been evaluated thoroughly by expert groups in the area, just not suggestions. I should point out I am not going to try to defend these in any way, but there have been guidelines and recommendations made.

Q: Lennette

I would like to make several points. First of all in answer to that last statement. The WHO Committee, let me point out that if you look at the list of members of that expert committee, there was no one there with an opposing point of view so that in my estimate there was present some degree of bias.

Now, let's get back to another aspect, and that is I think the environmental science people have gotten off under a false premise. It is assumed that since there has been no increase in bacteria-initiated outbreaks over the years, and with an increasing population and an incrasing amount of human waste, there must be a problem, and ergo it must be viruses. It remains to be proved, or at least shown, that viruses pose a problem.

Now in answer to what was raised by Dr. Rycheck earlier; yes, we have gone beyond the stage of no more than one viral particle per 100 gallons of drinking water. We have now passed the one virus over 500 gallon stage and the last I heard was one per 1000 gallons. Eventually, you are going to have a situation akin to the old saw about Salk polio vaccine: when safety problems were encountered it was pointed out that the only way you can be sure the vaccine is safe is to run the whole batch through the testing gamut, leaving you with zero ml for the populace. Surely there must be some rational point at which you have got to stop.

The other question I want to ask is: if you have a standard proposed, how do you explain Table 3 when you find echoviruses and yet no disease?

A: Gerba

Was a thorough epidemiological investigation conducted? In none of those cases could you really say that a thorough epidemiological investigation has occurred or was done. Those are costly evidence. Thus, you have no evidence that disease did not occur.

Q: Lennette

So what do these data mean?

A: Gerba

The data indicate that the population is being exposed to these viruses.

Q: Lennette

No it doesn't. It means that the population was excreting these viruses.

A: Gerba

Right, but there is exposure by water route through their drinking water. That is the point that we are trying to make here. I think we may be losing the perspective sometimes of what we are trying to accomplish in something perspective of environmental virology. Right now we are trying to determine the exposure of the population, the epidemiological investigation, if it can be done, and I doubt whether you can ever prove it epidemiologically. I am not going to try to defend it because I'm not an epidemiologist. You have to realize what we are trying to accomplish and that is trying to determine if there is really the exposure to the populations and should we be concerned that the health of the population is being effected or should we even be concerned because the viruses are present. We have to document the occurrences of viruses in ground water and in drinking water before we can further develop our premise and our concerns along those routes. If we don't have documentation, if we don't gather the data, we have no premise for going any further.

Q: Lennette

Well, this brings us to the point that Dr. Morrison mentioned, where does one draw the line on finding fewer viruses in larger amounts of water? We have to relate what we find to matters of human health. If we don't find a disease problem, then why do we spend money to get better and better techniques to detect the one viral particle? That is the question that I ask. There isn't enough money in this country to do all of the things that are proposed. And I think we have reached a point of absurdity when we say in print that viruses are pathogenic for man and therefore there should be no virus in any water with which man comes into contact.

A: Gerba

I am not trying to argue that point.

Q: Lennette

No, but I am being the devil's advocate. I think we ought to get some of these things out on the record and not pussyfoot around.

Q: Cabelli

Dr. Gerba, I presume you are not implying causality between the disease outbreak and the enteroviruses isolated from the water.

A: Gerba

No, but we are presenting some indication from the serological studies that some individuals in the population exposed to the water were also infected with the viruses in the water.

Q: Cabelli

Since the viruses were present in the population and being shed into the water, infection may have come about by some other route of transmission.

A: Gerba

That is a possibility.

Q: Sobsey

I think our discussion has finally reached the critical point taht we always seem to come around to when discussing viruses in water. That is, we have to keep reminding ourselves that these viruses can be transmitted in a number of different ways besides water. Direct and indirect person-to-person contact are the most important routes of exposure to enteric viruses, particularly within households or other situations where people are in close contact. There are other routes, including: the waterborne route, food-borne routes and possibly other environmental routes. The problem is trying to quantitatively determine which of these possible routes are significant. Because we here are concerned about ground water and other waters, we are predisposed to considering the waterborne route as a possible source of exposure to enteric viruses. However, our epidemiological tools, and even our virus detection tools, make it very difficult, perhaps at this point impossible, to quantitatively sort out which routes of exposure are the most significant in a particular situation. We have evidence of waterborne disease outbreaks due to such viruses as Norwalk-type agents and hepatitis A. Common source waterborne outbreaks of viral disease due to these agents do occur. However, we lack virus methods to detect these particular viruses because they are extremely difficult to isolate in the laboratory. It is very possible that when someone pumps a thousand liters of water through a virus-concentrating filter Norwalk virus and hepatitis A are recovered and wind up in the final concentrate samples. The problem is that these viruses will not be detected because our cell culture techniques make it difficult if not impossible to find them. What we find instead are other enteric viruses that don't appear to be significant causes of waterborne disease. We are faced with a dilemma. My appeal has always been to get the epidemiologists to help us find situations where we can try and sort out the significance of the various routes of enteric virus exposure. How significant is direct and indirect person-to-person transmission versus waterborne transmission, versus foodborne transmission. Are there situations where the role of water is significant? Clearly, outbreaks indicate that sometimes it can be, but there are many other situations not associated with explosive outbreaks where water may also be significant. Here is where a problem exists, because we don't have good epidemiological tools or even virological tools to get quantitative information on such situations.

Q: Wilson

Along these same lines it is most interesting to point out the certain lack of communication in that we have not been informed of Baylor's biological results in the Georgetown outbreak. We had epidemiologic data that fits very nicely with what they found. It explains some of the things that we could not explain. But if we are going to be honest with ourselves and talk about interdisciplinary cooperation I think it is important to realize that it can get sticky. There are state, federal, local health authorities, private universities, other governmental agencies, but I think this is a classic example of where there was the opportunity we might have even done other kinds of sampling in terms of collecting specimens from ill and well persons which did not happen. And I think when I talk about Georgetown I have a couple of extra slides that I was going to cut out for brevity but I think I will talk about data that suggests coxsackie B3 could have been an etiologic agent for this outbreak. You could have looked more intensively. Our epidemiologic tools were not necessarily that crude but we have to know in advance what we are doing in order to look for something.

WATERBORNE OUTBREAKS RELATED TO CONTAMINATED GROUND WATER REPORTED TO THE CENTERS FOR DISEASE CONTROL, 1971-1979

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ABSTRACT

Surveillance of outbreaks of waterborne disease is jointly conducted by the Centers for Disease Control (CDC) and the Environmental Protection Agency (EPA). Waterborne outbreaks are reported to the CDC and the EPA by state and local health departments and by water supply agencies; the data are tabulated annually. Previous CDC reports have specifically discussed the characteristics of waterborne outbreaks related to contamination of ground water. This report summarizes CDC surveillance data for the years 1971-1979 on waterborne outbreaks in public water systems where ground water contamination was reported to be the primary system deficiency responsible for the outbreak.

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INTRODUCTION

Surveillance of outbreaks of waterborne disease is jointly conducted by the Centers for Disease Control (CDC) and the Environmental Protection Agency (EPA). Waterborne outbreaks are reported to the CDC and the EPA by state and local health departments, and by water supply agencies; the data are tabulated annually. Previous CDC reports (1-5) have specifically discussed the characteristics of waterborne outbreaks related to contamination of ground water. This report summarizes CDC surveillance data for the years 1971-1979 on waterborne outbreaks in public water systems where ground water contamination was reported to be the primary system deficiency responsible for the outbreak.

Methods

An outbreak of waterborne disease is defined as an incident in which 1) 2 or more persons experience similar illness after consumption of water and 2) epidemiologic evidence implicated the water as the source of illness. In addition, a single case of chemical poisoning constitutes an outbreak when laboratory studies indicate that the water is contaminated by that chemical.

For the purpose of analysis, the water systems are classified as community, noncommunity or individual according to definitions set forth in the Safe Drinking Water Act (PL 93-523) of 1974. A community water system (previously referred to as "municipal") is defined as one that serves at least 15 service connections used by year-round residents or that regularly serves 25 year-round residents. A noncommunity water system (previously referred to as "semi-public") is one to which the public has access but which does not satisfy the criteria for a community system. We refer to community and noncommunity systems collectively as public water systems. Individual systems are those that supply one or a few private residences and usually derive water from wells or springs. Outbreaks occurring in individual systems will not be considered in this presentation.

The EPA Office of Drinking Water compiles statistics on the number and characteristics of all public water systems in the United States. Although this activity began in 1976, 1979 is the first year for which the compilation of data is reasonably complete as evidenced by only a small increase in the number of systems when compared to 1978. All of the rates in this report are therefore based on the 1979 statistics.

In Appendix I and II we present two waterborne outbreaks related to a contaminated ground water source to illustrate the major points of emphasis in this report.

Outbreaks

There were 226 disease outbreaks related to public water systems reported in the United States for 1971-1979; 87 of these outbreaks were in community systems and 139 were in noncommunity systems. Outbreaks were reported significantly more frequently from community than noncommunity systems (Table 1). Ninety-five (42%) of the outbreaks reported from public (community and noncommunity) systems were attributed to contamination of the ground water source while 60 (27%) involved contamination of a surface water source. In contrast to all waterborne outbreaks, those related to contaminated ground water and surface water were more likely to be reported from noncommunity systems (Table 2). Outbreaks related to raw water source contamination were more likely to be reported from systems with a surface water supply than systems with a ground water supply (Table 3).

Table 1
Reported Waterborne Disease Outbreaks
in Public Water Systems
United States, 1971-1979

Type of System	Number of Systems	Number of Outbreaks	Outbreak Rate/10,000 Systems
Community	53494	87	16.3
Noncommunity	133946	139	10.4

X^2=10.5 RR=1.6 p=.001

Table 2
Reported Waterborne Outbreaks in Public
Water Systems Due to Contamination of the
Raw Water Source,
United States,
1971-1979
I. Comparison of Systems

Water Source	Noncommunity System			Community System				
	Number of Systems	Outbreaks	Rate*	Number of Systems	Outbreaks	Rate*	RR	p-Value
Surface	3739	32	85.6	6283	28	44.6	1.9	.015
Ground	130207	80	6.1	47211	15	3.2	1.8	.043

RR=Relative Risk

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Table 3
Reported Waterborne Outbreaks in Public
Water Systems Due to Contamination of the
Raw Water Source, United States, 1971-1979
II. Comparison of Raw Water Sources

	Surface Water Source			Ground Water Source				
Type of System	Number of of Systems	Outbreaks	Rate*	Number of Systems	Outbreaks	Rate*	RR	p-Value+
Community	6283	28	44.6	47211	15	3.2	13.1	<.0001
Non-community	3739	32	85.6	130207	80	6.1	13.9	<.0001

RR=Relative Risk

The number of waterborne outbreaks related to contaminated ground water reported annually ranged from 4 in 1971 to 20 in 1976 (Figure 1), and these were reported from 29 states and Puerto Rico (Figure 2). Thirty-five (37%) of the ground water associated outbreaks were reported from Pennsylvania. There was no peak seasonal incidence for ground water-associated outbreaks involving community water systems. In contrast, ground water-associated outbreaks involving noncommunity systems occurred mainly during the months of June-August (Figure 3).

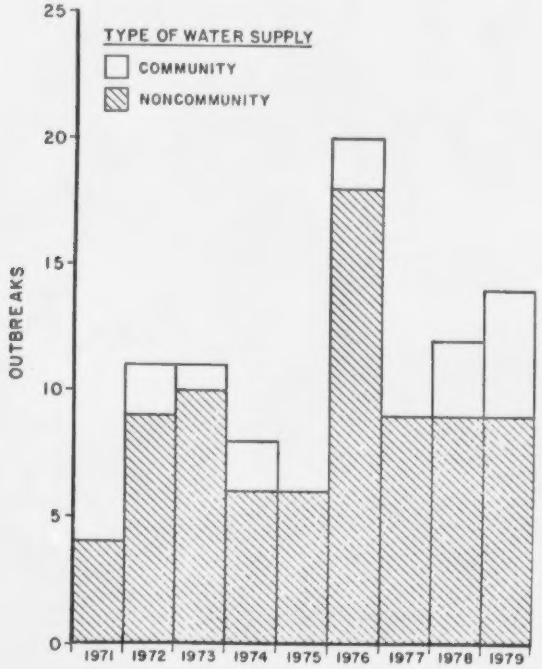
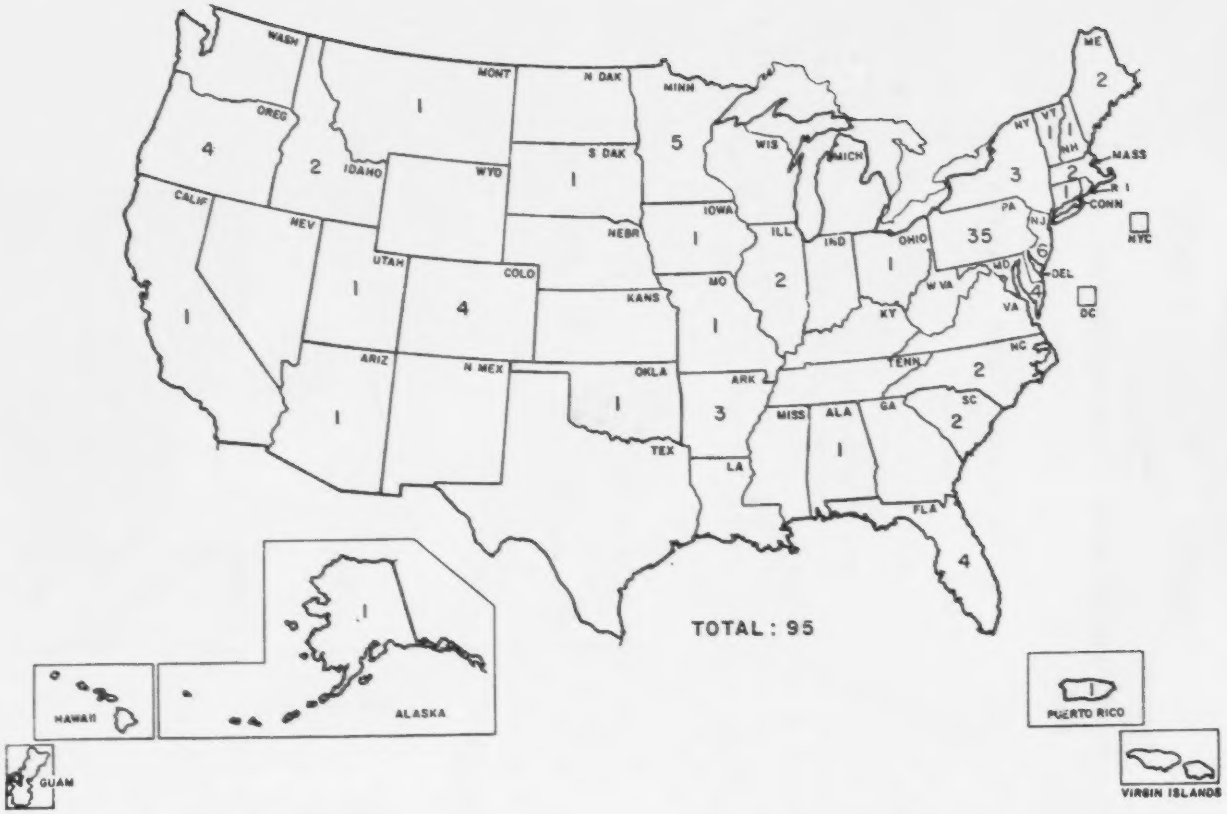


Figure 1. Waterborne Disease Ourbreaks Related to Ground Water Contamination Reported to CDC, 1971-1979


WASH
OREG 4
CALIF 1
IDAHO 2
NEV
MONT 1
WYO
UTAH 1
ARIZ 1
COLO 4
N MEX
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NEBR
KANS
OKLA 1
TEX
MINN 5
IOWA 1
MO 1
ARK 3
LA
WIS
ILL 2
MICH
IND
OHIO 1
KY
TENN
MISS
ALA 1
GA
FLA 4
SC 2
NC 2
VA
W VA
MD
DEL 4
PA 35
NJ 6
NY 3
VT 1
NH 1
ME 2
MASS 2
R I
CONN 1
NYC
DC
TOTAL: 95
ALASKA 1
HAWAII
GUAM
PUERTO RICO 1
VIRGIN ISLANDS

Figure 2. Waterborne Disease Outbreaks Related to Ground Water Contamination Reported to CDC, 1971-1979

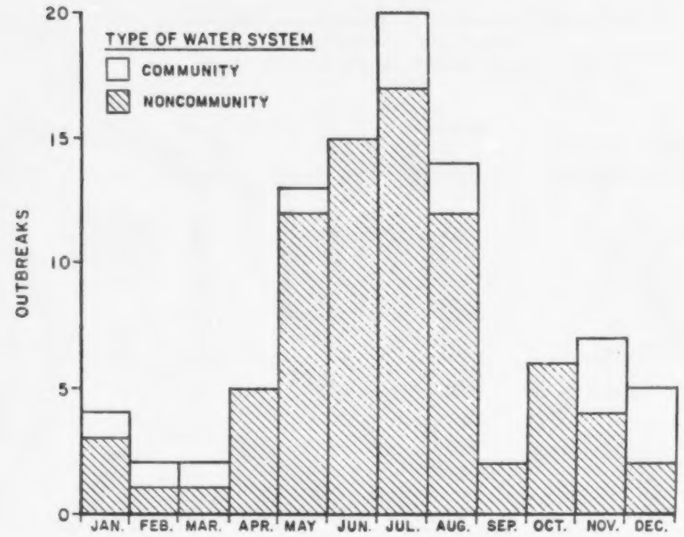

Figure 3. Reported Ground Water-Associated Disease Outbreaks, by Month of Occurrence, United States, 1971-1979

Fifteen outbreaks were reported associated with contaminated ground water supplying community water systems serving from 31-40,000 people (median 2950). Of the 80 ground water-associated outbreaks involving noncommunity systems, 34 (43%) involved systems serving parks, recreational areas or summer camps while 17 (21%) were associated with water systems serving restaurants (Table 4). Forty-four percent of these outbreaks in noncommunity systems involved exposure of fewer than 500 persons each. Four outbreaks, however, occurred in noncommunity systems each serving 1000 persons or more and therefore accounted for 54% of all persons exposed in noncommunity ground water related outbreaks (Table 5).

Table 4
Type of Establishment
Reported Noncommunity Ground
Water-Related Outbreaks
1971-1979

Type of Establishment	Number of Outbreaks	(%)
Park, recreational area, summer camp	34	(43)
Restaurant	17	(21)
Motel, country club, resort	9	(11)
School	4	(5)
Other	11	(14)
Unknown	5	(6)
	80	(100)

Table 5
Reported Noncommunity Ground Water-Related
Outbreaks 1971-1979
Classified by Number of Persons Exposed

Numbers of Persons Exposed	Number of Outbreaks	% of Outbreaks	% of Persons Exposed
<100	15	19	4
100-199	12	15	9
200-299	8	10	9
300-499	7	9	15
500-999	2	2	8
1000+	4	5	54
Unknown	32	40	-
Total	80	100	99

Coliform counts performed on raw water samples collected in ground water related outbreaks were elevated in all but one instance (Table 6). Where chlorination status was known, 8 of 10 community systems compared to only 29 of 69 noncommunity systems were reported to be chlorinated (p=.039, two-tailed Fisher's exact test).

Table 6
Water Quality Findings in
Reported Ground Water-Related Outbreaks*,
1971-1979

	Type of System	
	Community	Noncommunity
Coliform count elevated	10	64
Coliform count normal	0	1
Coliform count unknown	3	14

*Excluding 2 community and 1 noncommunity outbreak related to chemicals.

The source of ground water contamination was known for only 16 (17%) of all ground water related outbreaks (Table 7). In nearly all instances where the source of contamination was unknown, raw water contamination from nearby septic tank drain fields were incriminated.

Table 7
Source of Ground Water Contamination
for Reported Community and
Noncommunity Outbreaks
1971-1979

Source of Contamination	Number of Outbreaks	(%)
Not documented or unknown	79	(83)
Documented sewage contamination of well	8	(8)
Documented sewage contamination fissured rock or limestone aquifer	5	(5)
Surface water contamination of well	3	(3)
Total	95	(99)

There were no significant differences in the relative frequency with which bacterial or viral pathogens were associated with ground water and surface water related outbreaks (Table 8). *Giardia lamblia* was, however, isolated significantly more frequently in outbreaks related to contaminated surface water than in outbreaks related to contaminated ground water (Table 8). The estimated attack rate for illness in ground water related outbreaks ranged from 5% for hepatitis to 53% for Norwalk-like agent (Table 9).

Table 8
Etiology of Reported Waterborne Outbreaks
Due to Contamination of Raw
Sources

	Source of Water			
Etiologic Agent	Ground Water		Surface Water	
	Community System	Noncommunity System	Community System	Noncommunity System
Bacteria				
Shigella	4	8	1	2
Salmonella	1	2	0	1
Campylobacter	0	0	1	0
Enterotoxigenic *E. coli*	0	0	0	1
Virus				
Hepatitis	1	3	3	2
Norwalk-like agent	0	4	0	2
Parasites				
Giardia+	1	1	14	9
Other				
Chemical	2	1	0	0
Unknown	6	61	9	15

*outbreaks per 10,000 system-years +X^2=33.0 RR=18.2
p<.0001 comparing surface water rates to ground water rates.

Table 9
Estimated Attack Rates for Ground
Water-Related Outbreaks 1971-1979 By Type of
Enteric Pathogen Isolated*

Pathogen	Number of Outbreaks	Persons Exposed	Cases	Attack Rate (%)
Shigella	10	10877	4287	39
Salmonella	3	2362	349	15
Hepatitis	2	1032	55	5
Norwalk agent	2	511	271	53
Giardia	1	65	11	17
Unknown	34	13006	3993	31

*Excludes outbreaks related to chemicals and those with unknown attack rates.

Discussion

The limitations of these surveillance data must be appreciated in order to avoid misinterpretation. The number of outbreaks of waterborne disease reported to CDC and EPA clearly represents a fraction of the total number that occur. Because of incomplete or delayed investigations, the waterborne hypothesis could not be substantiated directly in all instances. In all the reported outbreaks, however, it was the most logical explanation. Caution must also be used in interpreting the reported number of outbreaks attributed to a given type of water system or attributed to a given etiologic agent. Large outbreaks and outbreaks of serious illness may be more likely reported to health authorities and investigated. Similarly, if interest exists in a given pathogen (i.e., Norwalk virus), outbreaks suspected of being associated with the pathogen may be more likely to have microbiological confirmation. Given these limitations, we report our observations based on the waterborne outbreaks reported to CDC from 1971-1979.

Our most striking finding was the lower rate of reported outbreaks associated with ground water contamination compared to the outbreak rate for systems using surface water. This lower rate was observed for both community and noncommunity systems. Although this observation must be interpreted with caution, it supports the concept that ground water may be less likely to be contaminated than surface water.

In contrast to outbreaks due to factors other than raw water contamination, noncommunity systems had a significantly higher rate of reported outbreaks than community systems. Surveillance artifact may play a role in this observation. For example, an outbreak which results from a defective distribution system may be more likely to be identified and reported in a community system than in a small noncommunity system. An alternative explanation is that noncommunity systems may be more likely to tap a ground water supply subject to microbial contamination. If the noncommunity system is unchlorinated, the chances of an outbreak occurring if the ground water source is contaminated is increased . The ground water associated noncommunity outbreaks had a peak occurrence in the summer months in settings such as summer camps, parks, and recreational areas. These systems have an abnormal demand placed upon them by large numbers of visitors during specific periods of the year and in some instances cannot meet such demands. No such seasonality was seen for ground water associated outbreaks in community water systems.

Excluding outbreaks related to chemicals, increased numbers of coliforms were noted for all but one outbreak for which data were available. Increased numbers of coliforms were found in raw water samples in all of the Norwalk agent-associated outbreaks as well as those outbreaks related to bacterial pathogens.

Documentation of the source of ground water contamination was infrequent. For those few instances where adequate documentation exists, sewage contamination of a well by a nearby septic tank drain field and sewage contamination of a fissured rock or limestone aquifer were the two most commonly observed defects. More emphasis should be placed on documentation of the source or raw water contamination in these outbreaks in order to insure that aquifer contamination is not widespread.

Giardia lamblia was almost exclusively associated with surface water source contamination. In the two instances of _Giardia_ contamination of a ground water source, either surface flooding of a well or the tapping of a shallow aquifer directly adjacent to a river were noted. The estimated pathogen specific attack rates observed in ground water associated outbreaks were either equal to or lower than those reported for other common source outbreaks due to these pathogens (6-9).

Ground water remains our safest source of drinking water. Care must be taken, however, to avoid contamination of aquifers and to document the extent of contamination when it occurs. The need to monitor and document outbreaks related to ground water contamination will increase with the increasing demand placed on our ground water resources.

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Appendix I

An Outbreak of Gastroenteritis in a Summer Camp in Pennsylvania, 1978

On July 27, 1978 an outbreak of gastroenteritis was reported from a summer camp in northeastern Pennsylvania. The cases were characterized by abdominal pain (80%), nausea (73%), and vomiting (53%). Headache (47%), diarrhea (38%), and chills (38%) were also prominant findings. The median duration of illness was 2 days.

Review of the infirmary records revealed 73 cases of gastroenteritis during the first session of the summer camp. This is approximately 10 times the rate reported from the previous year. A sharp increase in cases began 48 hours after the arrival of the second session campers (Figure 1). As determined from questionnaire data, the attack rate in the second session was 61.5% (120/195). Food was not incriminated. However, consumption of 5 or more glasses of water or a water containing beverage called "bug juice" was significantly associated with illness (Table 1). A telephone survey of 5 neighboring summer camps revealed no increase in the number of gastroenteritis cases reported during July 1978 relative to July 1976 or 1977. A diagram of the summer camp is presented in figure 2. One well supplied water for the camp residential areas and kitchen (Figure 1). Water from this well was chlorinated and stored in the holding reservoir; this reservoir was inadequately covered. At only 1 point did a water line and sewer line cross; the water line lay approximately 1 meter above the sewer line. Fluorescein dye flushed into a toilet located 15 meters from this potential cross-connection was not detected in water samples collected over a 72 hour period from faucets in the kitchen and in living areas located at the ends of the water distribution system. Residual chlorine levels were not routinely monitored and no residual free chlorine was detected in water samples taken from kitchen faucets on July 27. Water samples taken from outlets at the end of the distribution system and from kitchen faucets and unchlorinated well water contained 2 to 18 fecal coliform bacteria per 100 ml of water.

Laboratory studies of stools from 10 patients and 10 controls revealed no bacteria pathogens. Three of 3 paired sera specimens, however, showed a 4 fold or greater rise to Norwalk agent by radioimmunoassay.

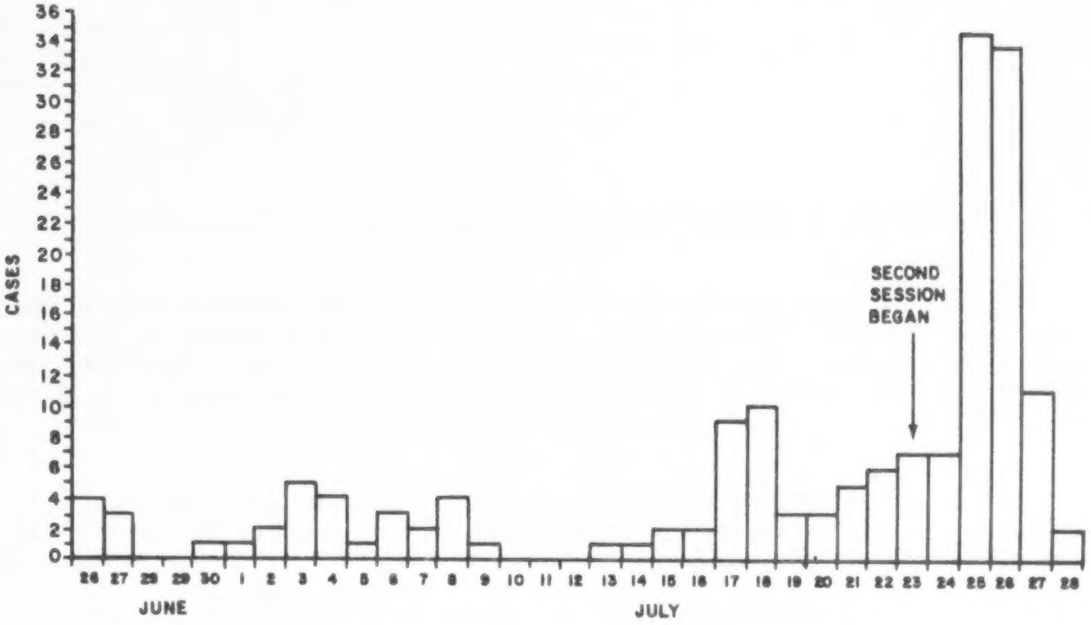

Figure 1. Gastroenteritis cases, by date of patient's visit to infirmary, Monroe County, Pennsylvania, June 26 - July 28, 1978.

TABLE 1
Location of Campers by Sex and Age Group, Summer 1978

Location	Sex	Age Group (Yrs.)
The Hill	Girls	10-16
The Line	Boys	6-9
The Row	Girls	6-9
The Village	Boys	10-16

Comments

This outbreak is typical of ground water-associated outbreaks occurring in noncommunity systems. Frequently multiple deficiencies are documented; for example, the reservoir and the distribution system had deficiencies which could have led to microbial contamination of the water system. Contamination of the ground water was documented by an elevated fecal coliform count in raw well water, and coupled with inadequate chlorination was responsible for this outbreak. It was not possible to demonstrate direct sewage contamination of the aquifer.

Serologic evidence implicated the Norwalk agent as the etiologic agent for this outbreak. In the four reported Norwalk outbreaks elevated coliform counts have been documented, suggesting that the presence of increased numbers of coliforms may be a satisfactory marker for the presence of this agent in raw water.

Appendix II

Gastrointestinal Illness in Georgetown, Texas - 1980

On June 16, 1980, CDC was informed of a possible outbreak of gastrointestinal illness in the city of Georgetown, Texas. A preliminary investigation was then conducted by the Texas State Department of Health revealed that 72% of respondents in a survey conducted in the central shopping area of Georgetown had been ill during the preceding 4 weeks. Predominant symptoms were diarrhea (90%), abdominal cramps (64%), nausea (36%), fever (30%), vomiting (24.1%); the number of new cases had peaked on June 13, and declined rapidly thereafer. No shared events could be identified for ill persons. CDC was invited to conduct a more extensive investigation.

A preliminary survey was conducted at an industrial plant outside Georgetown. A questionnaire was administered to second shift employees asking whether they had been ill since June 1, and if so to indicate whether they had had any of a list of symptoms; a case of gastrointestinal illness was defined as diarrhea and/or 2 or 4 other symptoms, including nausea, vomiting, abdominal cramps, and fever. Individuals were also asked about the source of water in their home. There was a statistically significant association between illness and living in a home served by the Georgetown municipal water system; since June 1, 12 (75%) of 16 individuals who used Georgetown municipal water in their homes had had an illness which meet our case definition, compared with 11 (15%) of 73 whose houses were not on the Georgetown municipal system (p<.0001, Fisher's exact test, two-tailed). There was also a statistical association between illness and Georgetown water consumption for individuals not served by Georgetown water. Of 11 persons who were ill and lived in an area not served by the Georgetown system, 4 (36%) recalled drinking water in Georgetown during the 2 weeks preceding the survey compared with 2 (3%) of 63 well persons (p=.0036, Fisher's exact test, two-tailed).

Water for the city is supplied by deep wells; there is a cluster of 4 wells in the central part of the city, with additional wells in San Gabriel Park and at the municipal airport (Figure 1). The airport well, park well and central city wells are chlorinated independently. In general, property within the city limits (including all restaurants, schools, and parks) is supplied by water from the central city and park wells (Area 1); mixing of waters from wells in the 2 locations is fairly complete, although areas immediately adjacent to the park receive a higher percentage of park well water, while property near the central city wells receive a higher percentage of central city well water. Residential areas north of the city, including the subdivision of Serenada (Area 3) near the airport are supplied by water from the airport well. Mains connect the airport and park and city systems but a combination of pressure gradients and one way valves effectively prevent mixing. Residential areas west of the city, known as Oakcrest Estates and Greenridge (Area 3), are supplied by a mixture of water from the airport well and the park and central city wells; one way valves prevent back flow of this mixed water into the airport or park and central city systems. Outside these areas (Area 4) water is obtained from private wells or other municipal systems. In order to asess the magnitude of the outbreak in Georgetown and to determine if the deficiency of the water supply system could be epidemiologically incriminated, a stratified, randomized telephone survey of the city was conducted between June 21-June 23. Four strata based on the water distribution system for the city were selected. Adults answering the telephone were asked about the presence or absence of diarrheal illness since June 1 and were asked the average amount of water consumed daily and whether they had drunk any water in Georgetown outside of their homes since June 1.

Eighty-one percent of individuals living within the Georgetown city limits (area 1) had had a diarrheal illness since June; the frequency of symptoms were similar to those found in the preliminary community survey. For individuals in area 1, the attack rate did not differ significantly for those who drank water only at home and those who drank water outside the home Table 1). Attack rates were significantly lower for individuals who lived outside area 1 and who drank water exclusively at home: the attack rate in Oakcrest Estates and Greenridge (area 2) was 48% ($p<.05$); the attack rate of the airport area (area 3) was 32% ($p<.005$); and the attack rate in persons not on the municipal water system (area 4) was 11% ($p<.001$). For individuals who remembered drinking water outside the home since June 1, there was no significant difference in attack rates among areas (Table 1).

Further evidence incriminating the public water system was obtained from a nursing home survey and a survey of persons attending a convention in the city at the time of the outbreak. Two nursing homes in Georgetown supplied data on the number of residents who had had a diarrheal illness since June 1. Nursing home A received a mixture of park and central city water; nursing home B is adjacent to San Gabriel Park, and its water comes primarily from the park well (Figure 1). At nursing home A the attack rate was 99%, a significantly higher attack rate then the 40% attack rate found in nursing home B. Ninety-one percent of questionnaire respondents who attended a convention in Georgetown at the time of the outbreak reported that since June 1 they had had a diarrheal illness which met our case definition. The number of new cases peaked on June 12 and declined thereafter with no evidence of a second peak. There was an association between water consumption and illness.

TABLE 1

Gastrointestinal illness attack rates* by area of residence and place of water consumption, †Georgetown, Texas, June 1980.

	Drank Water only at home			Drank water outside home		
Area	Ill	Well	Attack rate	Ill	Well	Attack rate
1 (within city limits)	63	17	79%	64	13	83%
2 (Oakcrest estates and Greenridge)	13	14	48%	43	17	72%
3 (airport)	12	25	32%	28	11	72%§
4 (not on municipal water system)	7	54	11%	42	10	81%§

*Data obtained in telephone survey.

†Place of water consumption not specified for 38 persons.

§Attack rate significantly higher for individuals drinking water outside the home--$p < .0001$, Fisher's exact test, two-tailed.

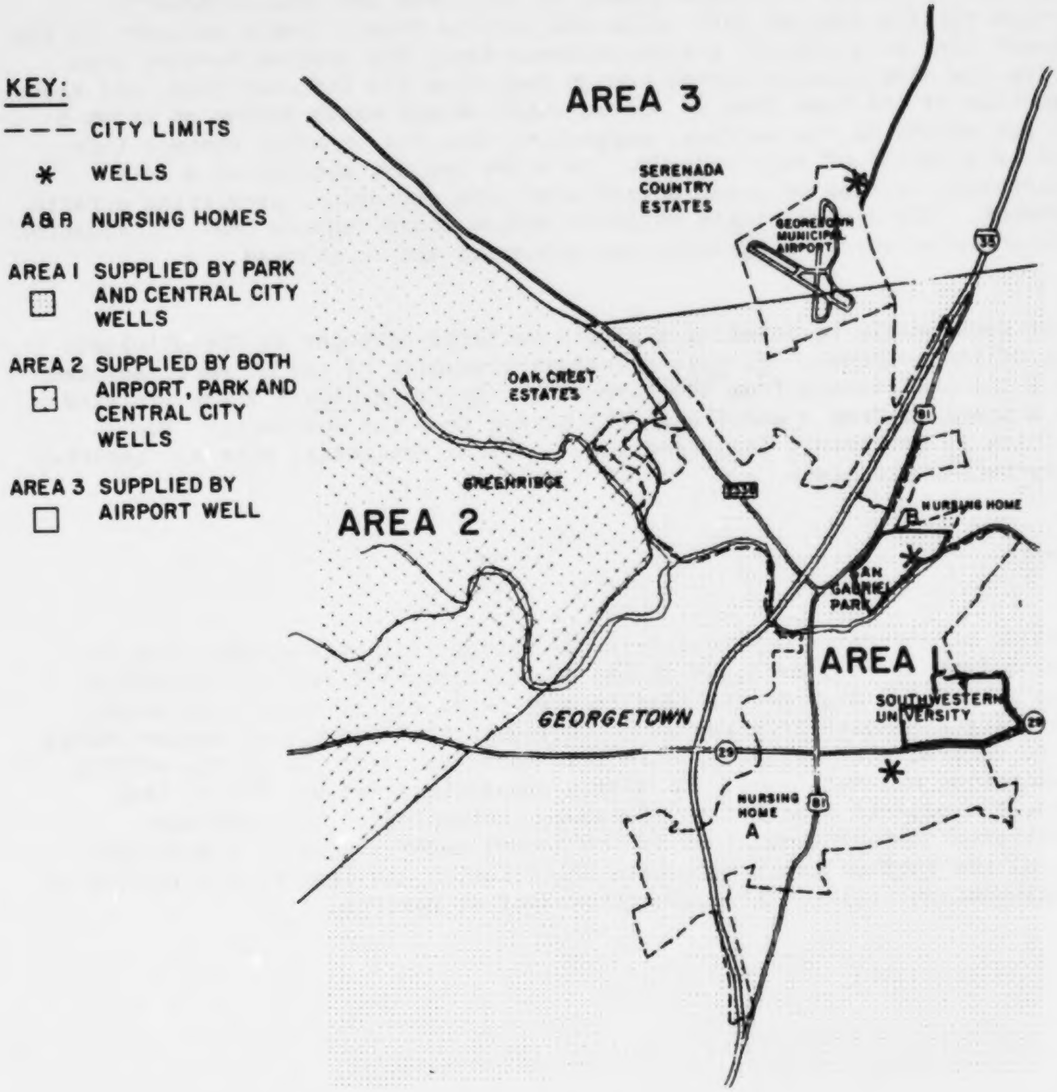
KEY:
CITY LIMITS
WELLS
A&B NURSING HOMES
AREA 1 SUPPLIED BY PARK AND CENTRAL CITY WELLS
AREA 2 SUPPLIED BY BOTH AIRPORT, PARK AND CENTRAL CITY WELLS
AREA 3 SUPPLIED BY AIRPORT WELL
AREA 3
SERENADA COUNTRY ESTATES
GEORGETOWN MUNICIPAL AIRPORT
35
OAK CREST ESTATES
81
GREENRIDGE
2338
AREA 2
NURSING HOME
B
SAN GABRIEL PARK
AREA 1
SOUTHWESTERN UNIVERSITY
GEORGETOWN
29
29
NURSING HOME
A
81

Figure 1. Gastrointestinal illness by area of patients' residence, Georgetown, Texas, June 1980.

A review of bacteriologic data for the water system collected since January 1979 indicated that water samples were usually obtained on only 1 day of the month, rather than at regular intervals during the month. No samples from the municipal system were submitted for bacteriologic analysis between May 20 and June 16; water samples drawn on June 16 from the distribution system showed no growth, but raw well water collected on the same day was contaminated (a specimen from wells not numbered, one of the central city wells, showed "heavy confluent growth of coliforms and noncoliforms"). Chlorine for the central city wells was applied from a single cylinder to the influent line of a 250,000 gallon concrete tank; the nearest booster pump feeding the distribution system was 25 feet from the influent line, and visual inspection of the tank from an access hatch showed water moving at about 5 feet per second on the surface, suggesting that the chlorine contact time could be a matter of only seconds. Chlorine was not applied at a rate proportional to flow or synchronized with pump operation, permitting erratic treatment. The use of single cylinder system would require that chlorination be interrupted when the cylinder was exhausted and/or changed.

We were unable to identify a single bacteria pathogen as the etiologic agent of the outbreak. *E. coli* organisms producing LT and ST were isolated from 2 ill individuals from the community. *Shigella boydii* 2 was isolated from a specimen from 1 additional ill person from the community. The remaining 14 specimens, including the 4 control specimens, were all negative for bacterial pathogens.

Comments

Using attack rates obtained from the random telephone survey, one can estimate that as many as 8,000 of the 10,000 persons living in Georgetown became ill with a diarrhea illness from drinking contaminated city water. Bacteriologic samples from raw water indicated that the ground wawter source for the central city wells may have been contaminated. The source of this contamination was not found. No single etiologic agent was found, thus fitting the classic description of "sewage poisoning". This outbreak demonstrates that contamination of the ground water source of a municipal water system coupled with inadequate chlorination can lead to an outbreak of gastrointestinal illness affecting thousands of persons.

HEALTH CONSIDERATIONS - OUTBREAK DATA: RICKEY WILSON

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: Salvato

Just to place the number of outbreaks within a realistic context, the number of outbreaks reported are the results of the reports you have received from state and local health departments. What ratios do you feel the numbers reported bear to the probable actual number of outbreaks?

A: Wilson

Well, we would suggest that Pennsylvania probably represents a fairly decent system of reporting. They probably don't investigate every potential outbreak but they do try to investigate most outbreaks. We have no evidence that the quality of the water systems or water sources in Pennsylvania are any better or any worse than any other state, so I think that you're probably looking at at least a ten-fold difference between reported and actual. That is a guess, and if I had to pull a number out probably we are looking at an order of magnitude.

Q: __________

What data do you have on diseases from these organisms?

A: Wilson

There is a real problem with _Yersinia_; it can be found in well water unassociated with human disease. We investigated one outbreak of _Yersiniosis_ thought to be water-related and _Yersinia_ was isolated from the water. It was not the same serotype of

Yersinia found in the patients. You can isolate it from water readily, especially in northern United States and Canada. As to date we have not had any clinical or epidemiologic evidence to suggest that Yersinia from these water sources are related to disease. That is really all we know right now on coxisidiosis in water.

Q: Rycheck

On Table 9 where you have your ground water related outbreaks, 1971-79, you indicate there that over half have unknown etiology. Would you like to comment on that?

A: Wilson

I think that one may postulate a viral etiology. We are in the process retrospectively reviewing clinical data and looking at the clinical syndromes in these patients to see if they fit the "Norwalk pattern" or not. It may well be, for instance, that Norwalk or Norwalk-like viruses cause a significant proportion of these outbreaks. But again, what this usually means is there has not been a very aggressive pursuit of etiologic agents, neither from a patient standpoint or water quality standpoint. We don't know if there are, for instance, any toxigenic E.coli in most of these instances. One of the real problems we had is if it is not Salmonella and if it is not Shigella and if the people don't turn yellow, up until about two or three years ago you just didn't know the cause of the outbreak, then the Pennsylvania camp outbreak in 1978 was followed by a flurry of activity in documenting Norwalk outbreaks or Norwalk-like outbreaks related to water. So that again it appears to be something new but I don't think it is. It may be a big piece of the "unknown" pie.

Q: Gregg

Rickey, would you tell us what the nature of CDC's collaborative work with EPA is on these outbreaks? I noted that you mentioned this earlier. I think the audience might like to know.

A: Wilson

We basically maintain a very close working relationship with EPA in that they call us when they hear about something and we call them. In those instances where state and local authorities are not able to provide the technical expertise needed for evaluation of the water system and where it is mutually agreeable between the state and EPA, then we have EPA input into the evaluation of the water system. In the case of Georgetown, for instance, we had EPA there evaluating the water system looking for the possible deficiencies. I think that we have tried and I think EPA will agree, we try to maintain a very close working relationship. It is not always easy and communication can be difficult. But I think the point is that we rely on EPA for the technical expertise and we try to go in and evaluate what has happened, what have been the health effects, and then see if we can mesh these two data sets to make sense out of what is going on and I think that that is very important.

Q: Little

I have just one question about Table 9, you mention that the type of enteric pathogen isolated. Does this refer from the cases or from the patients?

A: Wilson

From patients, sir. These are what we isolated from patients. In some cases we do have pathogens from water. In most cases you don't. For one of the Salmonella outbreaks, Salmonella was actually obtained from the water and in three of the Shigella outbreaks Shigella was actually cultured from the raw water source. These were the agents from the patients, the causitive agent of the outbreak. The attack rates and generally speaking the only point I was really trying to make here is that generally speaking the attack rates were somewhat lower than if you compared literature with foodborne outbreaks, and other non-source outbreaks of the same nature.

Q: Gerba

Doesn't the greater number of outbreaks in non-community water supplies really reflect your ability to recognize these because you usually have a limited number of individuals whereas these would be very difficult to recognize in the larger community?

A: Wilson

Well, I think that there are certainly surveillance artifacts that biases any of these systems. I think that it depends on the kind of outbreak. As I pointed out in the paper, in a community system you are probably much more likely to pick up this sort of classic "sewage poisoning" from cross connections because of the number of people involved. In the non-community system, you pick them up because the population served has temporarily increased. You've got summer camp, you've got 200 or 300 kids there nad it is very hard for the camp nurse to ignore it when 120 kids within two days of each other all get diarrhea and show up at the infirmary. There is no doubt about it there are selection biases in how we come about finding out about these various kinds of outbreaks. And I agree, we have to be very, very cautious in interpreting these data for that very reason.

Q: Morrison

Would you mind commenting on observations you have made where the indigent native population does not seem to show a high infectivity rate whereas transients, visitors, recreational people might show high rates.

A: Wilson

It is usually pretty hard to sort that out. In most of the outbreaks we have done, the number of permanent residents has been very small; therefore it becomes very hard to make the kinds of analyses we would like. And the other problem we run into is the age dependent phenomena. It isn't quite fair to compare apples and oranges and compare 300 nine-year olds in terms of what illness they get after exposure with 50 forty-year olds that happen to live in the area. And you often don't have the right populations to compare with each other. I think we certainly have postulated in the past that some of these cases if you take careful histories you find that you get the statement, "Oh Yeah. We've had diarrhea when we were younger drinking this water." You get antecdotal things but in terms of really good data you say they are immune to whatever it was we don't have. Probably the closest thing we come to is tourist groups where we have had the Puerto Vallarta outbreak last winter with multiple tour groups going to various hotels and through a fairly elaborate work-up it appeared to be water related, with nice dose responses and yet no evidence that the local people drinking the same water had any increased incidence of diarrhea illness. This suggests that their exposures to the causitive agents occurred much earlier and that at the time of the outbreak they were immune. This is about the best recent experimental nature we have at the time.

EPIDEMIOLOGICAL APPROACH TOWARD DETERMINING ENDEMIC TRANSMISSION OF WATERBORNE DISEASE

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ABSTRACT

There are several barriers which can be, and generally are, imposed sequentially to reduce the health risks involved from soil disposal of wastewaters. They are (i) treatment and disinfection of the wastewaters, (ii) filtration, adsorption and die-off of the agents during transport through the soil, and (iii) treatment and disinfection of the raw drinking water source. The effectiveness of these barriers is usually determined by routine monitoring programs against water quality standards or "removal requirements" for several physical chemical and biological parameters. Included are the free chlorine residual, total and/or fecal coliform densities, and the "standard plate count" in the water. These standards are derived -- at least in theory -- from available information on case and outbreak reports and their retrospective epidemiological analyses, the findings from prospective epidemiological studies, and/or indicator-pathogen density relationships.

Three questions can be raised concerning the status quo of current water treatment, standards and monitoring practices. Are the coliform indicators currently used adequate? What circumstances, if any, are likely to produce endemic cases of waterborne disease which are not detected as case and outbreak reports? Are such cases being sustained at present?

This paper discusses work done to date relevant to the three above questions. The author suggests that more rigorous treatment and disinfection of sewage and more stringent drinking water standards will be required if future research on the adequacy of currently accepted indicator organisms is negative.

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EPIDEMIOLOGICAL APPROACH TOWARD DETERMINING ENDEMIC TRANSMISSION OF WATERBORNE DISEASE

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As noted earlier in this conference, the land application of domestic wastewater carries with it the risk of infectious disease via several routes of transmission. The one of most concern at this meeting is the ingestion of ground waters contaminated with infectious agents transported through the soil. In addition, nearby surface waters used for recreation or as raw sources of drinking water may also become contaminated via stormwater runoff or the lateral, sub-surface movement of the wastewater through the soil.

There are several barriers which occur or can be imposed to reduce the health risks involved in the land application of sewage. They are (i) treatment and disinfection of the wastewater, (ii) filtration, adsorption and die-off of the agents during transport through the soil, and (iii) treatment and disinfection of the raw drinking water source. The effectiveness of these barriers is usually determined by routine monitoring programs against water quality standards or "removal requirements" for several physical chemical and biological parameters. Included are the free chlorine residual, total and/or fecal coliform densities, and the "standard plate count" in the water. These standards are derived -- at least in theory -- from available information on case and outbreak reports and their retrospective epidemiological analyses, the findings from prospective epidemiological studies, and/or indicator-pathogen density relationships.

Thus, in one sense at least, the subject of this conference, potential health effects consequent to the land disposal of domestic wastewaters, can be considered as a special case of the more general question, the effectiveness of the existing drinking water standards.

There can be no question that modern methods of water treatment and disinfection have reduced the risk of drinking-waterborne infectious disease. Moreover, it is generally concluded that the promulgation and enforcement of the microbiological standards for drinking water have also contributed to this reduction. This follows from the assumption that the levels of these indicators in the water is a proper reflection of the degree of fecal contamination and/or the "adequacy" of treatment and disinfection (hence, the risk of infectious disease). Nevertheless, outbreaks of drinking water-associated infectious disease continue to occur (1,2), although, in most instances, it can be shown that the application of the microbiological standards, rather than the standards themselves, was at fault (3). However, this has not been true of all the outbreaks.

CURRENT PRACTICES AND STANDARDS

The reduction in case and outbreak reports notwithstanding, the status quo with regard to water treatment, standards, and monitoring practices could be questioned from two points of view. First, do the existing microbiological standards, and even the specified levels of treatment and disinfeciton, permit an "appreciable" number of sporadic (endemic) cases of infectious disease which, by their very nature, go undetected as case and outbreak reports? Moreover, if the nature and number of cases were known, would they constitute an "unacceptable risk". Second, are treatment and disinfection deficiencies and the health consequences thereof more widespread than is reflected in case and outbreak reports or violations of the microbiological limits. These two questions can be paraphrased by three others. Are the coliform indicators currently used adequate? What circumstances, if any, are likely to produce endemic cases of waterborne disease which are not detected as case and outbreak reports? Are such cases being sustained at present?

Adequacy of Microbial Indicators and Standards

The two microbial indicator systems most frequently used in the United States to monitor the quality of drinking water are total coliforms and the "standard plate count". The U.S. Environmental Protection Agency standards for total coliforms examined by the mENDO method are a quality limit of one coliform per 100 ml of water and an action limit of four per 100 ml. Both the quality and action limits for the "Standard Plate Count" are 500 organisms per 100 ml (4).

The coliform indicator systems have been criticized as not surviving environmental stress, including disinfection (chlorination) and residence in sewage and aquatic environments as well as some of the "hardier", viral, enteric pathogens (5-7). Several alternative indicator systems, such as enterococci, *Clostridium perfringens* spores, acid-fast bacilli, yeasts and coliphages, probably better simulate the survival characteristics of the viral pathogens (8-10) (Table 1, Figure 1). At least two of the indicators, *Streptococcus fecaelis* (a part of the enterococcus population) and *C. perfringens*, were proposed as water quality indicators along with *Escherichia coli* at the turn of the century (8). Nevertheless, they are seldom used in the United States, although they continue to be used in Europe (11). Prescott et al (8) present a number of arguments against the use of these two systems. However, these arguments are supported by data obtained with insensitive methods, come from comparisons of their densities to those of the coliforms (a "no-win" comparison), and relate to the risks of illness from salmonellae and shigellae rather than to those from the more "environmentally stable" viral pathogens. Moreover, no information was available comparing the indicator densities to an independent arbitor, epidemiological data. The last three systems noted above, acid-fast bacilli, yeast and coliphage, are not fecal indicators. However, with disinfected drinking waters, at least, the survival properties of the indicator may be so dominant as to negate the need for a fecal source.

TABLE 1. REDUCTION IN INDICATOR DENSITIES FOLLOWING CHLORINATION

Indicator	Log_{10} Density/100 ml or Reduction			
	Prechlorination		Reduction[a]	
	b	c	b	c
Fecal coliforms	5.992	4.914	4.763	1.921
E. coli	5.745		4.726	
Enterococci	4.310		3.576	
C. perfringens	4.284		0.733	
Acid-fast bacteria		3.246		0.340
Yeasts		2.894		1.303
Coliphage	3.114		1.692	

[a] Residual chlorine values averaged 0.8 - 1.0 mg/liter.

[b] Mean values from 9-16 sets of values from a sewage treatment plants in Rhode Island

[c] Mean values from nine-ten sets of values from a sewage treatment plant in Illinois (After Englebrecht et al, 1979) (9).

Factors Likely to Produce Sporadic (Endemic) Cases

Several factors, individually or in combination, would tend to produce sporadic cases rather than reported outbreaks of disease from waters which meet the existing microbiological standards. They are (i) relatively benign symptomatology, (ii) appreciable transmission by other routes, (iii) a high infectious dose coupled with low levels of the agent in the water, (iv) the requirement for predisposing conditions for disease (opportunism), (v) a population which is predominantly immune to the agent in question, and (iv) agents which are appreciably more resistant to environmental stress than the indicators.

One or more of these factors is operative with most of the microbial agents responsible for cases of waterborne enteric disease. However, for the present discussion, only two groups of viral agents will be considered, the agents of gastroenteritis (the human rotavirus and the parvo-like viruses) and hepatitis A virus. All the factors with one possible exception, the infectious dose, could be operative with the former group. However, the presumably low infectious dose required for infection may be overridden by the prevalence of immunity, at least with the rotavirus. Moreover, a "non-specific gastroenteritis" is the most commonly reported waterborne disease (3), although bacterial agents such as *Campylobacter* (12) *Yersinia enterocolitica* (13) and enteropathogenic *E. coli* (14) have been identified as the etiological agents in several such outbreaks. The symptomatology for infectious hepatitis is certainly not benign, but there are subclinical forms of disease. In addition, there is an appreciable annual incidence of infectious hepatitis (15); and the route of transmission has not been established for many of the cases, presumably because they have occured sporadically. The retrospective cohort analysis of some sporadic cases of infectious hepatitis produced some evidence of an association with shellfish consumption (16). However, this approach probably would produce equivocal findings in a search for sporodic cases of infectious hepatitis -- or, for that matter, any infectious disease -- associated with drinking water.

Evidence for Sporadic (Endemic) Cases

To the author's knowledge there is no direct evidence that sporadic cases of infectious disease are occurring from the consumption of drinking waters which meet the current standards. Cases of gastroenteritis were detected in the prospective epidemiological studies conducted by Peterson and Hines in the 1950's (17). Most of these were associated with the consumption of water from systems which would not be acceptable at this time (Table 2). However, the extent to which the cases in community F were also due to the drinking water could not be determined because there were no controls.

Implications from Bathing Beach Studies

An analogous situation existed with regard to swimming-associated illnesses. That is, a single, prospective, epidemiological study conducted in the 1950's (18) suggested that sporadic cases of gastroenteritis in children were associated with swimming in the sewage-polluted waters of a stretch of the Ohio River; and the bacteriological results were extrapolated to derive the current fecal coliform guidelines (19,20). A second series of prospective epidemiological-microbiological studies was conducted under the aegis of the United States Environmental Protection Agency. These results showed that there was an appreciable rate of sporadic cases of gastroenteritis associated with swimming in sewage-polluted waters that were well within the existing indicator guidelines (21,22). Moreover, it is from the indicator-illness relationships obtained from this study that one might speculate on existence of sporadic cases of gastroenteritis from the consumption of some drinking waters which meet the existing guidelines and standards.

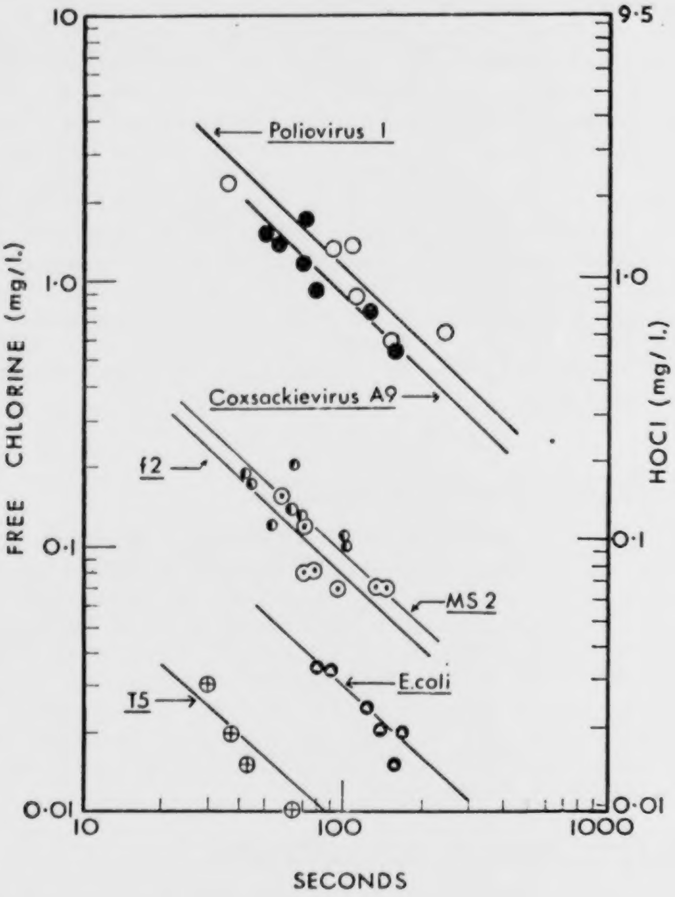

Fig. 1. Concentration-time relationship for 99 per cent inactivation of animal viruses, bacterial viruses, and the reference bacterium (Escherichia coli ATCC 11229) by hypochlorous acid at 5°C and pH6. (5)

TABLE 2. THE RELATION OF WATER QUALITY TO GASTROINTESTINAL ILLNESS INCIDENCE IN 6 ROCKY MOUNTAIN COMMUNITIES

Community	Individuals Surveyed	Individuals Ill	Attack Rate,%	% of 1 ml Water Sample Portions Positive
A	199	34	17.1	100.0
B	285	40	14.0	90.0
C	352	42	11.9	72.5
D	313	31	9.9	0.0
E	375	34	9.1	2.5
F	335	25	7.5	1.7

From Peterson and Hines (17).

The experimental design and results of these studies have been published (21,22). Those design features which are relevant to the present discussion were as follows. The initial studies were formulated to determine which symptoms were both swimming-associated and polluted-related and the indicator whose densities were best correlated to the swimming-associated rates for those symptoms. Four populations (and subpopulations thereof) were examined, swimmers (heads immersed in the water) and non-swimming beach-goers at "barely acceptable" and "relatively unpolluted" beaches. The bathing water densities of a number of potential, microbial water quality indicators were determined; and regression analyses against the rates of the swimming-associated, symptoms were used to determine the best indicator and, with the findings from subsequent studies, to define the illness-indicator relationships (criteria).

Four findings from the overall program have relevance to the foregoing discussion. First, only gastrointestinal (GI) symptoms and the highly credible portion thereof (HCGI) were both swimming-associated and pollution-related. An individual was recorded as positive for GI symptoms if he reported vomiting, diarrhea, stomachache or nausea. He was reported positive for HCGI symptoms if he reported vomiting; diarrhea with a fever or severe enough for him to remain home, remain in bed or seek medical advice; or stomachache or nausea with a fever. Second, enterococci were the "best" indicators; E. coli was a poor second; and there was little, if any, correlation of the rates of swimming-associated GI symptoms to the mean total or fecal coliform densities (Figure 2). Third, appreciable rates of swimming-associated HCGI symptoms, gastroenteritis, (about 10/1000 swimmers) were associated with relatively low mean enterococcus or even E. coli densities (about 10 per 100 ml) in the water (Figure 3). Moreover, this corresponds to the ingestion of 1-5 enterococci or E. coli when the quantity of water ingested during a swimming experience is considered (it is estimated at 10-50 ml). This is less than the daily ingestion of coliforms allowable under existing drinking water standards. Fourth, the age distribution of the GI and HCGI symptoms and the comparison of the indicator-illness relationships obtained from the U.S. studies, Cairo tourists at Alexandria, Egypt beaches, and the Alexandria residents suggests that immunity plays an important role in the epidemiological picture (21, 22).

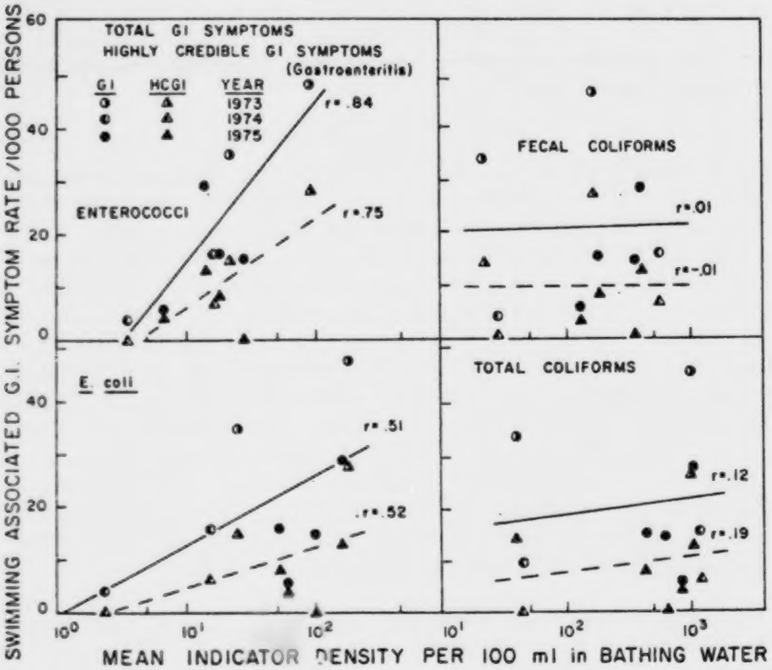

Fig. 2. Swimming-associated rates of gastrointestinal symptoms against the mean indicator densities in the bathing water. Each point comes from the trials conducted at a given beach during a given year of the three year New York City study.

A PROSPECTIVE EPIDEMIOLOGICAL DRINKING WATER STUDY

The rather speculative extrapolations made in the preceding section are not presented to suggest that the existing standards, and hence any involved risks of infectious disease, are not acceptable. However, the web of circumstantial evidence -- possible indicator defects, the conditions for sporadic cases to occur,

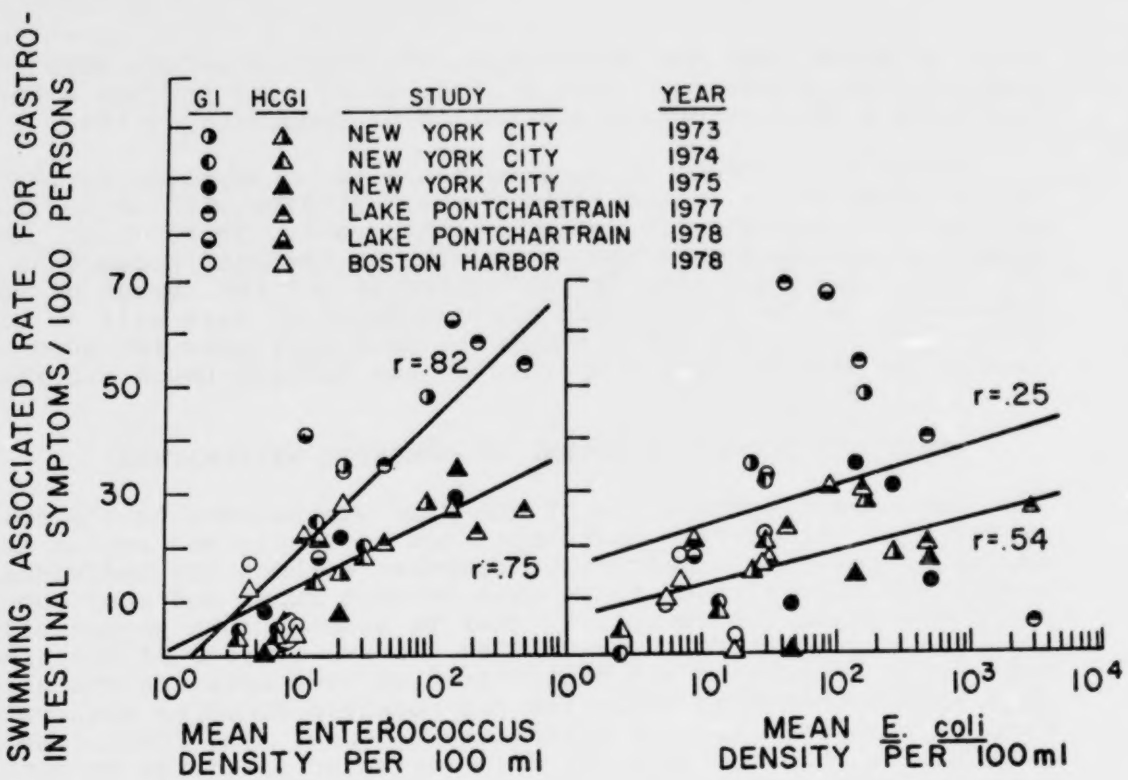

Figure 3. Swimming-associated rates for GI symptoms against the mean enterococcus and E. coli densities in the water. Data from all U.S. studies. Definition of highly credible GI symptoms given in text. The rationale for clustering the trials and the values for the points are given in reference 22.

and the implications of the bathing beach studies -- appears sufficient enough to warrent the conduct of a prospective epidemiological-microbiological study to determine what risks are being accepted from the consumption of drinking waters which meet current standards. Such a study would also provide a vehicle for a more objective evaluation of indicator systems.

Finding a control group comparable to or approaching that of the nonswimmers in the bathing beach studies will be a difficult but not necessarily insurmountable task. In addition, more extensive monitoring of the water will be required because of the longer duration of a "trial" interval and the number of homes involved. On the other hand, the gathering of data will be simpler; and the participants should be more available for serological or medical follow-up than in the bathing beach studies.

RELEVANCE TO SOIL DISPOSAL OF DOMESTIC WASTEWATERS

The direct application of domestic wastewaters to the soil is not new. Subsurface discharge from cesspools and septic tanks is one of the historical sewage disposal methods for individual homes, and it continues to be used in most rural and many suburban communities. Moreover, it must be assumed that potentially impacted groundwaters to be used as the raw sources of drinking water will be treated and disinfected to meet existing standards. This notwithstanding, there are two possible concerns with regard to the potential for infectious disease. The first, the possibility that the existing drinking water standards permit an unacceptably high incidence of sporadic cases of waterborne infectious disease (notably gastroenteritis), has been considered earlier. The second is that, because of the quantities of sewage which could be applied to the land and factors involved in its transport through the soil, coliform indicator deficiencies such as those observed in the bathing beach study may be even more pronounced and the risk of disease more understated. This, in turn, could require more rigorous treatment and disinfection of the sewage and potentially impacted gound waters against more stringent standards using more appropriate indicator systems.

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EPIDEMIOLOGY APPLIED TO ENDEMIC TRANSMISSION: VICTOR CABELLI

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: Morrison

Dr. Cabelli, on the bathing beaches, particularly in New York and Boston Harbor, were there sources of contamination or pollution, other than human? In other words, were there industrial discharges or similar discharges?

A: Cabelli

The sewage from combined systems impacted the beaches in the New York City, Boston Harbor and Egyptian studies. The sources of pollution, other than stormwater overflows following heavy rainfall, were less defined in the Lake Pontchartrain study. We are looking into this. Beyond that I can only answer surely there must have been industrial sources, and it is possible since we were dealing in two of the studies, certainly the Boston Harbor study and the New York City study, with combined sewer systems that there could have been animal waste as well.

Q: Morrison

Might I ask if you think that possibly this is what gave you a better correlation for enterococci rather than strictly E. coli?

A: Cabelli

This is a possibility.

Q: __________

Is there a significant difference between the survival of any of these enteric viruses in sea water than in contrast to regular drinking water?

A: Cabelli

I think Dr. Sobsey can better respond to this question.

Q: Sobsey

In general, enteric viruses tend to survive less well in sea water, or other saline waters than in fresh water. Although I hesitate to make a blanket statement that would hold in all cases, most of the reports in the literature suggest that there is more anti-viral activity in sea water or water mixed with sea wter than in fresh water.

Q: Lennette

Do your studies provide a direct or indirect relationship of the indicator densities to the levels of viruses in the water?

A: Cabelli

When we designed the epidemiological studies, the only viruses in the bathing water which could be enumerated were the enteroviruses. In the absence of well documented outbreaks of waterborne, much less swimming-associated, enteroviral disease, we could not justify the cost of making such measurements, especially in view of the poor methodology available at the time. More important, since the etiology of the most common waterborne disease (a non-specific gastroenteritis) was unknown and possibly mixed, our approach (like Stevenson before us) was to look for a direct relationship of illness among swimmers to the densities of a number of indicators in the water.

Q: ___________

Was there any correlation to the reduction in raw sewage discharges by New York City to the epidemiological and microbiological findings?

A: Cabelli

It would seem so. Over the three years we conducted our studies there, 1973-1975, there were decreases in the mean enterococcus densities at the Coney Island beach and corresponding decreases in the rate of swimming-associated gastroenteritis. Furthermore, it would appear from our on-going microbiological studies that the mean enterococcus have decreased even further.

Q: Audience

One of the real problems that may occur as far as sporadic effect of beaches is that a significant number of young children, especially, will actually go to the bathroom in the water.

A: Cabelli

That is certainly a possibility, and I would think that this could be a significant factor in disease transmission for small bodies of water with little water exchange. I do not think it was a significant consideration in any of the studies we conducted.

A REVIEW OF INFECTIVE DOSE DATA FOR ENTEROVIRUSES AND OTHER ENTERIC MICROORGANISMS IN HUMAN SUBJECTS

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ABSTRACT

The dose response in humans has been studied for enteric bacteria, animal parasites and viruses. An illness end point was determined in the reviewed studies for bacterial pathogens and an asymptomatic infectivity end point was determined for amoebae and viruses. Infectivity was determined by fecal shedding of the organisms and/or a specific antibody response. Studies were selected for review that evaluated response over a dosage range so that an indication of the probability of a response could be determined.

The enteric bacteria yielded the widest dosage range requirement to produce a response. Salmonella spp. required 10^5 to 10^8 cells to produce a 50% attack rate in healthy adults. Three species of Shigella were capable of producing illness in a significant percent of dosed subjects with 10 to 100 cells. Similarly, amoebic infections were produced with Entamoeba coli and Giardia lamblia dosed in gelatin capsules at the level of 1 to 10 cysts. Enteric viruses also were found to produce infection at very low doses. One cell-culture-detectable unit of polioviruses (vaccine strains) produced infection in infants and children when the virus was delivered directly to the gastrointestinal tract. In a recent study, poliovirus 1 (Sabin strain) was administered directly into the oral cavity of 2-month-old infants to determine the dose response under a more normal exposure route. The 50% infective dose was determined to be 72 $TCID_{50}$. Statistical analysis of the data indicated that 1% of the subjects could be infected with 20 $TCID_{50}$. A more recent study conducted with 142 healthy adult volunteers, determined the dose response to a wild enterovirus, echovirus 12, following oral ingestion. Statistical analysis of these data indicated that 35 PFU represented a 50% infective dose and that 1-2 PFU would likely infect 1% of susceptible subjects.

These data show that the infective dose for some members of all three types of enteric organisms can be extremely low, ≤ 10 detectable units, for healthy subjects.

INTRODUCTION

Mankind's relationship with microorganisms is a dynamic one that defies absolute characterization. An effort is made to divide the microbial world into pathogenic and non-pathogenic organisms. However, few would disagree that a broad "gray area" exists in this classification. Two rather recent experiences will illustrate this point.

Only thirty years ago, this country experienced a record high number of cases of poliomyelitis. In the early 1950s, 21,000 cases of paralytic polio were occurring annually primarily in healthy children and young people. Yet for every paralytic case in children, serological data show that about 1,000 children experienced asymptomatic infections and shed high numbers in their stools (1). For the vast majority (99.9%) of these infected persons, the polioviruses were not pathogenic. A high percentage of asymptomatic infections as observed in this case is probably typical of most encounters with microbial pathogens.

A different experience seems to have occurred with giardiasis. Historically, the cysts of the protozoan, Giardia lamblia, were so frequently observed in stool specimens from asymptomatic persons that clinicians did not normally prescribe treatment to rid the intestinal tract of this organism. In 1970, two outbreaks of giardiasis occurred in American visitors returning from the Soviet Union. Symptoms included nausea, abdominal cramps, diarrhea, anorexia and fatigue. Cysts were identified in the stools of 32 patients and 5 asymptomatic persons associated with the outbreaks. Drinking water from a Russian municipal supply was believed to be the source of the organisms. Subsequently, numerous waterborne outbreaks of giardiasis have been documented. In fact, since 1977 Giardia lamblia has been the most frequently identified agent associated with waterborne disease in the U.S. (2). Apparently, a dramatic change in the host-parasite relationship with this organism has occurred in the U.S. during the past 10 years.

The theory of parasitism states that evolving symbiotic relationships are in the direction of decreasing injury to the host. The theory states that over time, parasitism (harm to host) gives way to commensalism (benefit to symbiont, no harm to host) and finally to mutualism (mutual dependence). There are examples of this progression. However, there appear to be examples of regression as well, recent U.S. experience with G. lamblia being a case in point. Therefore, it may be debated whether there is a consistent one-directional evolution in this relationship.

Nonetheless, there are many factors that affect the outcome of microbial exposure on an individual level. Host susceptibility is influenced by age, sex, nutritional and disease status and prior exposure. Pathogenicity and virulence of the organism is altered by mutation, selection and genetic transfer. Environmental factors may determine the route of exposure, persistence of the organisms and the level of exposure.

Acknowledging these factors, perhaps it is absurd to attempt to establish a minimum exposure level at which a generalized response to a specific microorganism may be predicted. The often used term "minimum infective dose" is a misnomer if it is considered in such an absolute sense. A public health conservative approach may constrain one to assume that single particles of known pathogens may cause an adverse response and that measures should be instituted to prevent this minimal level of exposure. However, economic and other practical restraints mitigate against an arbitrary acceptance of this position. Obviously, data are needed to form a basis for making relative risk judgments in regard to environmental exposures.

In considering host responses to microorganisms, a distinction is to be made between the two most common end points measured: infection and illness. Infection may be defined as multiplication of a microbial agent within a host with or without the production of disease. Asymptomatic infection is the desired relationship with a pathogen because immunity may be achieved without the health risk associated with the related disease, e.g., live virus vaccines. The occurrence of illness may be determined by the manifestation of a single pathogenic effect or a group of symptoms normally associated with an etiological agent.

Both end points have been utilized to study host-parasite interactions in human populations. However, investigators, institutions and the public in general are becoming increasingly reluctant to support studies designed to produce pathogenic effects in humans. Therefore, much of the recent microbial response data, especially the virus data, are obtained by the asymptomatic infectivity end point as determined by fecal shedding of the test organism or by the detection of a specific antibody response. Of course, the asymptomatic infective dose of a pathogen for one individual may produce disease in another. However, it is generally assumed that severity of response is proportional to the degree of exposure and in feeding studies that administer relatively low doses, to determine the minimum infectivity level, adverse effects in healthy subjects are rare. Nonetheless, infective dose is an important parameter in risk evaluations because infected persons may transmit viable organisms to others who may experience clinical disease. As an added precaution, the termination of infection can usually be insured by the administration of antibiotics and other antimicrobial drugs when bacterial or protozoal agents are studied. Virus studies are normally conducted with vaccine or very mildly-pathogenic strains since antibiotic control is not available for enteric viruses.

DOSE RESPONSE TO ENTERIC BACTERIA

The organisms of greatest concern in exposure to wastewater-contaminated environments are the enteric bacteria and viruses and the intestinal parasites. In the U.S., _Salmonella_ and _Shigella_ species are essentially the only enteric bacteria that have a recognized prevalence level sufficient to be of concern. Hornick _et al_. (3) have conducted dose-response studies in healthy adult male volunteers with _Salmonella typhi_, the etiological agent of typhoid fever. This study determined the number of organisms required to produce an illness end point. A positive response was determined when toxic symptoms of typhoid fever occurred, e.g., headache, malaise, anorexia and temperature of 103° F for 24 to 36 hours. At this point, the infection was interrupted by the administration of an antibiotic. Table 1 shows the dose response obtained with the oral administration of various densities of organisms suspended in 30 ml of milk. No symptoms of typhoid fever were observed in 14 volunteers who received 10^3 organisms. Half of the volunteers who ingested 10^7 organisms became ill.

TABLE 1. NUMBER OF _SALMONELLA TYPHI_ (QUAILES STRAIN) ORGANISMS REQUIRED TO PRODUCE TYPHOID FEVER IN HEALTHY ADULT MALE VOLUNTEERS (3)

Number of Viable Organisms Administered*	Dose Response No. Ill/No. Challenged (%)
10^3	0/14 (0)
10^5	32/116 (28)
10^7	16/32 (50)
10^9	40/42 (95)

*Organisms suspended in 30 ml of milk

Similar studies were conducted by McCullough and Eisele (4,5) to determine a salmonellosis end point for several other _Salmonella_ strains and species. Table 2 shows the results of these studies and indicates that a wide variation in cell numbers was required to produce illness. Six adult male volunteers ingested various densities of the organisms suspended in a glass of eggnog. Subjects were selected who had no indication of _Salmonella_ infections, i.e., no organisms in their stools and absence of high serum agglutination titers. Specific symptoms included abdominal cramping, nausea, diarrhea and low grade fever. The natural course of the illness was followed without specific treatment except when medically indicated. Two strains of *S. meleagridis* did not produce illness in the 12 volunteers who received 5,500,000 organisms. However, the ingestion of 1,700,000 cells of *S. bareilly* produced illness in 4 of 6 (67%) subjects. The lowest pathogenic dose observed with the eight organism studied was 125,000 cells of *S. bareilly*. One of 6 subjects became ill after ingesting this number of organisms.

TABLE 2. NUMBER OF *SALMONELLA* ORGANISMS REQUIRED TO PRODUCE CLINICAL ILLNESS IN HEALTHY ADULT MALE VOLUNTEERS (4,5)

	% of Volunteers Ill*			
Organism	0	15-20	30-50	65-85
	Dose x 10^6			
S. anatum, Strain I	.26		.58	
S. anatum, Strain II	24	45	67	
S. meleagridis, Strain I	5.5	24		50
S. meleagridis, Strain II	5.5	10	20	41
S. meleagridis, Strain III	1.5	7.7	10	
S. bareilly		.13	.70	1.7
S. newport		.15	1.4	
S. darby	6.4		15	

*Six volunteers normally exposed/dosage level; organisms administered in glass of eggnog

Shigella has been found to be a more virulent genus of enteric bacteria than *Salmonella*. Symtoms of shigellosis include diarrheal stools containing blood and mucus, abdominal cramps and high fever. A group comprised of federal and university investigators has conducted a series of *Shigella* feeding studies in inmates of a correction institution (6,7). Adult male volunteers were fed various types and strains of *Shigella* organisms suspended in milk. Inocula were prepared by diluting 24-hour agar plate cultures and the cell number confirmed by making pour plates of the inocula before and after each experiment was conducted. Illness was terminated in the volunteers by the administration of an antibiotic.

Results of studies with *S. flexneri 2a* and two strains of *S. dysenteriae 1* are shown in Table 3. All three organisms produced illness in a significant number of subjects fed 200 cells. One of 10 subjects fed 10 cells of *S. dysenteriae 1*, strain M131 became ill. This strain had been responsible for a dysentery pandemic in Central America during 1968-1970. The virulence and multi-drug resistance of this organism actually led to work on a live immunizing agent for this disease. Although not reported in detail, these investigators mention in an additional report the production of disease in a significant percentage of adult volunteers fed 10 to 100 viable cells of *S. flexneri 2a* and *S. sonnei* as well as *S. dysenteriae 1* (8).

A limited amount of dose-response data has been reported for three additional enteric bacteria that, on recent occasions, have been associated with waterborne disease in the U.S. and could be of health concern in wastewater exposure (9,10). *Yersinia enterocolitica* can produce in man an enteritis similar to salmonellosis. Szita *et al.* (11) have reported that illness was produced in a single volunteer fed 3.5 x 10^9 cells of the organism. Enteritis also has been recently associated with the ingestion of *Campylobacter jejuni*. One investigator experienced typical symptoms after ingesting 10^6 organisms, in 200 ml of milk, of a strain that had been isolated from a patient with diarrhea (12). Pathogenic and toxigenic strains of the common intestinal bacterium, Eschericha coli, have been associated with waterborne enteritis. Koya et al. (13) studied the illness response of E. coli enterotoxigenic strain 0-111 B4 in four male volunteers. No illness resulted in the subject fed 2.7 x 10^7 organisms. Mild diarrhea, abdominal pain and fever resulted in 3 subjects fed 5 x 10^7 to 10^9 organisms. DuPont et al. (14) studied two nontoxigenic invasive strains of E. coli and observed illness in 8 of 13 adult volunteers fed 10^8 cells. No clinical disease was apparent on ingestion of 10^6 cells unless an antacid was also administered. An additional enteric bacterium Vibrio cholerae, although no longer a significant pathogen in this country, remains as a major etiological agent in the developing countries. Studies with the Inaba 569B strain of V. cholerae have indicated that a dose of 10^8 cells is required to induce diarrhea in the absence of concomitant antacid administration (15). None of these studies except Koya et al. (13) and DuPont et al. (14) were designed to determine a minimal dose response. However, the limited data available indicate that all four organisms are comparable to Salmonella in the number of cells required to produce a pathogenic response. Shigella appears to be the only genus of enteric bacteria that can produce illness in healthy adults at relatively low exposure levels, i.e., ≤ 200 organisms.

TABLE 3. RESPONSE IN HEALTHY ADULT MALE VOLUNTEERS TO VARIOUS DOSES OF VIRULENT STRAINS OF SHIGELLA

Organism	Dose	No. Ill/No. Fed	% Ill	Ref.
S. flexneri 2a	180	9/36	22	6
	5,000	28/49	57	
	10,000	52/88	59	
S. dysenteriae 1 (Strain A 1)	200	1/4	25	7
	10,000	2/6	33	
S. dysenteriae 1 (Strain M 131)	10	1/10	10	7
	200	2/4	50	
	2,000	7/10	70	
	10,000	5/6	83	

DOSE RESPONSE TO ANIMAL PARASITES

Historically, amebiasis caused by the protozoan Entamoeba histolytica has been the most important environmentally transmitted disease of animal parasite origin in the U.S. Most infections are asymptomatic; however, its pathogenicity is well documented and the occurrence of cysts in the stool is always of concern. Rendtorff was interested in determining the dose-response of this organism in man, but felt it improper to purposely expose prisoner volunteers to this pathogen. He chose instead to determine the infective dose of a non-pathogenic amoebae: Entamoeba coli (16). Adult male volunteers that showed no amoebic infections on stool examinations were selected for this study. The volunteers were individually housed under specifically controlled environmental conditions during the 10-week study period to minimize extraneous infections. The cyst inoculum was obtained from volunteer donors and was separated from debris and other organisms by a flotation procedure and micromanipulation. Individual dosage levels were obtained by micromanipulator isolation of the cyst, a very accurate technique for obtaining low-dose numbers. Appropriate volumes of the cyst suspensions were placed in gelatin capsules and swallowed with 120 to 180 ml of tap water.

Table 4 shows the results of this study. Infection, as determined by repeated fecal shedding of cysts, was achieved in one of eight volunteers who supposedly ingested only one cyst. The increasing infectivity rate with doses of 10 (30%) and 100 (50%) cysts lends support to the authenticity of the single positive response (12.5%) on ingestion of one cyst. In addition the rigorous experimental design that included isolation of the volunteers, negative controls and a highly sensitive cyst counting procedure tends to lend credibility to the remarkable finding.

The reluctance of Rendtorff to conduct human feeding studies with E. histolytica was apparently not shared by Beaver et al. (17). They obtained cysts from an asymptomatic donor and fed adult volunteers a single dose ranging from 2,000 to 1,000,000 cysts. Unfortunately, the lowest dose used in this investigation produced infection in all volunteers (42 of 42) which precluded the determination of a minimal infective dose (Table 4). It should be noted that the authors concluded that no disease symptoms could be attributed to E. histolytica infections in these subjects.

TABLE 4. RESPONSE IN ADULT MALES TO VARIOUS DOSES OF AMOEBIC CYSTS

Cyst Type	Number of Cysts Given	No. Infected/ No. Fed	% Infected	Method of Adm.	Ref.
E. coli	1	1/8	12.5	gelatin capsule	16
	10	3/10	30		
	100	2/4	50		
E. histolytica	2000 to 4000	42/42	100	beverage suspension	17
G. lamblia	1	0/5	0	gelatin capsule	18
	10	2/2	100		
	100	2/2	100		

Although Giardia lamblia was not the important pathogen in the 1950s that it became in the 1970s, Rendtorff included this protozoan in his study of the E. coli model of amoebic infection (18). This early work represents the only human dose-response study conducted to date with this organism. Volunteers were fed either Giardia cysts alone or Giardia plus E. coli cysts in gelatin capsules. The inocula counts were determined by micromanipulator isolation as stated above. A single volunteer received one of three dosage levels of Giardia alone or the same dosage levels of Giardia and E. coli cysts. Table 4 shows the results of this study and indicates that a dose of 10 cysts or less is sufficient to produce infection in a high percentage of exposed susceptible individuals. It is likely that a significant percentage would be infected with a single cyst even though none of the 5 volunteers exposed to this level demonstrated infection in this study. Perhaps the apparently more virulent strain occurring in the U.S. today would be more infectious. However, the direct correlation of parasite virulence (as indicated by disease frequency or severity) with the exposure level required for a host response has not been adequately demonstrated. Interestingly, these data indicate that these animal parasites have about the same degree of infectivity as Shigella bacteria.

DOSE RESPONSE TO ENTEROVIRUSES

The last group of enteric pathogens to be considered, viruses, represents a somewhat more difficult agent to be safely studied in humans. Since viruses are obligate intracellular parasites, even the infectivity end-point requires the occurrence of the somewhat uncontrollable process of cell destruction, i.e., virucidal drugs are not available to interrupt the infectious process. The development of avirulent strains of polioviruses in the 1950s provided opportunity to conduct dose response studies with live vaccine strains with minimal risk of adverse responses. It also allowed such studies to be conducted with a subset of the population that appears to be more susceptible to natural infections, i.e., infants and children.

Koprowski and his colleagues at Lederle Laboratories conducted the first reported dose response studies with attentuated strains of polioviruses. Strain SM of poliovirus type 1 had been attenuated by rodent adaptation followed by successive passages of the virus in chick embryo and monkey kidney tissue culture. The virus was non-pathogenic for monkeys on intracerebral injection. As a component of the field trials of this potential live vaccine virus, Koprowski et al. (19) conducted a dose response study in children at a state institution. A total of nine children, who showed no antibodies to type 1 poliovirus, were given various doses of the virus suspended in polyethylene glycol 400 (0.5 ml) within a hard gelatin capsule. Each subject swallowed two capsules consuming at the same time 8 ml of milk. The dosage was determined by making 10-fold dilutions of a virus preparation titrated by the plaque technique using monkey kidney cell monolayers. Infection was determined by fecal shedding of the virus or by a specific antibody response.

The results of the study are shown in Table 5. Of course, the dose of 0.2 PFP is a dilution-determined average value and indicated that a single plaque-forming particle (PFP) would not occur in most doses. The calculated dose of 2 PFP produced virus shedding and an antibody response in 2 of 3 subjects. The investigators, aware of the implications of this remarkable finding, suggested that the following factors should be clarified before assuming that these data represented a practical occurrence (20): (a) interference in virus titration by the diluent thereby giving an inaccurately low value, (b) insensitive assay system that did not detect all the PFP that would be infectious for the subjects and (c) artifically high sensitivity due to delivery of the encapsulated virus directly to susceptible cells in the intestinal tract. Concurrent studies with an attenuated type 2 poliovirus indicated that 300 units of this virus were required for infection (20). However, titration was performed in intact mice yielding mouse paralytic doses 50%, a less sensitive quantitative procedure.

Poliovirus vaccine feeding studies were subsequently studied by others over the next few years (21). However, significant infection levels (>50%) were not reported with doses $<10^{3.5}$ tissue culture infective dose 50% ($TCID_{50}$) until the studies of Katz and Plotkin (22) in the mid-sixties. These investigators studied the dose response of poliovirus type 3, Fox strain in 22 premature infants in the nurseries of a general hospital. Within the first 48 hours of life, each infant was given a low dosage of the virus, suspended in 5 ml of Hanks' solution, directly into the stomach by gavage using a rubber oro-gastric tube. The tube was flushed with 10 ml of saline solution before removal. Infectivity was determined by fecal shedding of the specific virus type administered.

Results of this study are shown in Table 5. At the lowest dose administered, 1 $TCID_{50}$, 3 of 10 subjects were infected. The $TCID_{50}$ quantitation procedure is thought to be less precise than the plaque procedure. However, the investigators demonstrated a high degree of accuracy in dose titration. The lower dosage levels (2.5 and 1 $TCID_{50}$) were titered before administration to the infants by adding 0.1 ml aliquots of each inoculum to 50 cell culture tubes. The titration results yielded positive findings within 0.5 tubes of the statistically predicted number for each titer. A line fitted to a log probability plot of the dose-response data indicated that the 50% infective dose for the subjects was 4 $TCID_{50}$. These findings and the authors' conclusion that "a dose of any pathogenic virus sufficient to infect tissue culture would also be infectious for man" have been extensively cited.

TABLE 5. RESPONSE OF INFANTS AND CHILDREN TO LOW DOSES OF POLIOVIRUS LIVE VACCINE

Dose	No. Infected / No. Fed	% Infected	Method of Adm.	Virus Type (Strain)	Ref.
PFP*					
0.2	0/2	0	Gelatin	1 (SM)	19
2	2/3	67	Capsule		
20	4/4	100			
$TCID_{50}$†					
1	3/10	30	Gavage	3 (Fox)	22
1.5	3/9	33	Tube		
10	2/3	67			
16	0/2	0	Aqueous	1 (Sabin)	23
50	3/6	50	Susp.		
90	3/4	75			
160	3/3	100			

*plaque-forming particles

†50% tissue culture infective dose

Recent EPA-Supported Dose Response Studies

In an effort to obtain virus infectivity data by more normal exposure routes, EPA has funded an additional infant poliovirus feeding study at the University of Wisconsin (23). Infant patients of a private pediatric practice were recruited to receive a reduced dose of the commercial poliovaccine type 1 (Sabin) two weeks prior to the scheduled receipt of the full dosage. The commercial vaccine was diluted in sterile distilled water to the desired dosage and administered in 0.5 ml volumes to the oral cavity with a 1-ml syringe. Each infant was observed to detect expectoration so as to insure that the entire dose had been swallowed. Infection was determined by the shedding of virus in the stool within 10 days post inoculation. Viruses isolated in the stool were identified as polio 1 by specific neutralization test.

Thirty-two 2-month-old infants were fed doses of 7 to 280 $TCID_{50}$ of virus. Few infants were given the identical dose since experiments conducted at different periods with freshly diluted virus gave slightly different titers. Results obtained with multiple feedings of the same dose are shown in Table 5. In addition to the 2 infants fed 16 $TCID_{50}$, three more yielded negative findings at doses of 42, 27 and 7 $TCID_{50}$. Statistical analysis of the data estimated a 50% infective dose for the subjects of 72 $TCID_{50}$, considerably higher than the findings of Koprowski and Katz and Plotkin.

An additional study has been supported by EPA to obtain data on the infective dose in adults of a "wild" enteric virus. The virus, echovirus 12, used in the study had been isolated from an 8-year-old girl with erythema infectiosum (fifth disease). Previous volunteer studies had shown the virus to be a very mild pathogen normally producing asymptomatic infections with common-cold type symptoms occurring infrequently.

Healthy male students having no evidence of echovirus 12 infection were recruited from local colleges. Selected subjects were isolated, 2 to a room, from outside contact for 8 days, one day prior and 7 days after ingestion of the virus. Appropriate dilutions of the purified virus were suspended in 100 ml of distilled water. Volunteers ingested 100 ml of the virus suspension or 100 ml of sterile water (negative control) under a double blind experimental design. Neither the volunteers nor the investigative staff knew the contents of the inoculum. Health status of dosed subjects was monitored twice a day by a nurse and physician. Throat and rectal swabs were collected daily during the isolation period for virus assay. Blood specimens were collected on the day prior to inoculation and on days 6 and 26 for echovirus 12 antibody tests.

Preliminary results from this study have been reported (24) and are summarized in Table 6. At the lowest dose administered, 10 plaque-forming units (PFU), an infection rate of 19% was observed. None of the subjects became ill. Subsequent to this report, additional data have been obtained for a total of 108 subjects fed one of four concentrations of virus: 10, 30, 100 and 300 PFU (Schiff, personal communication).

TABLE 6. INFECTION RESPONSE IN HEALTHY YOUNG ADULT MALE VOLUNTEERS TO VARIOUS ORAL DOSES OF ECHOVIRUS 12 (24)

Dose (PFU)*	No. Infected/No. Fed	% Infected
10	6/32	19
30	2/7	29
100	14/21	67

*Plaque-forming units

Statistical analyses of these data and the data of Minor (23) are shown in Table 7. The 50% infective dose is easily calculated from the data. The 1% and 10% infective doses are less reliable because they represent extrapolations beyond the experimental data using statistical analysis. Experimental data in this dosage range are difficult to obtain due to the number of subjects required and the imprecision in viral quantitation. For example, at a mean of 1 PFU/inoculum volume, the virus follows a Poisson distribution which predicts that 37% of the inocula would contain zero PFU. Therefore, it is impossible to disinguish between a non-response in a large percentage of the subjects due to administration of no virus versus administration of sub-infective numbers, a major objective of a dose-response study. Under this condition, the experimental error may be greater than the extrapolation error from a statistical analysis. Figure 1 shows the dose response curve for echovirus 12 produced by probit analysis. The probit transformation was used because it is based on a normal tolerance distribution and the assumption of normality seems the most appropriate for this type of data. These data provide the strongest evidence yet available that a single detectable virus unit can be infectious for a portion of the population.

TABLE 7. ESTIMATES OF THE NUMBER OF CELL-CULTURE-INFECTIVE DOSES OF TWO ENTERIC VIRUSES REQUIRED TO PRODUCE INFECTION IN HUMANS WHEN INGESTED ORALLY

Virus	% Infected	Virus Dose	95% Confidence Limits	Ref.
		$TCID_{50}$		
polio 1	50	72	55-93	23
	10	39	24-63	
	1	20	7-52	
		PFU		
echo 12	50	35	21-64	24
	10	3	<1-7	
	1	.4	<1-2	

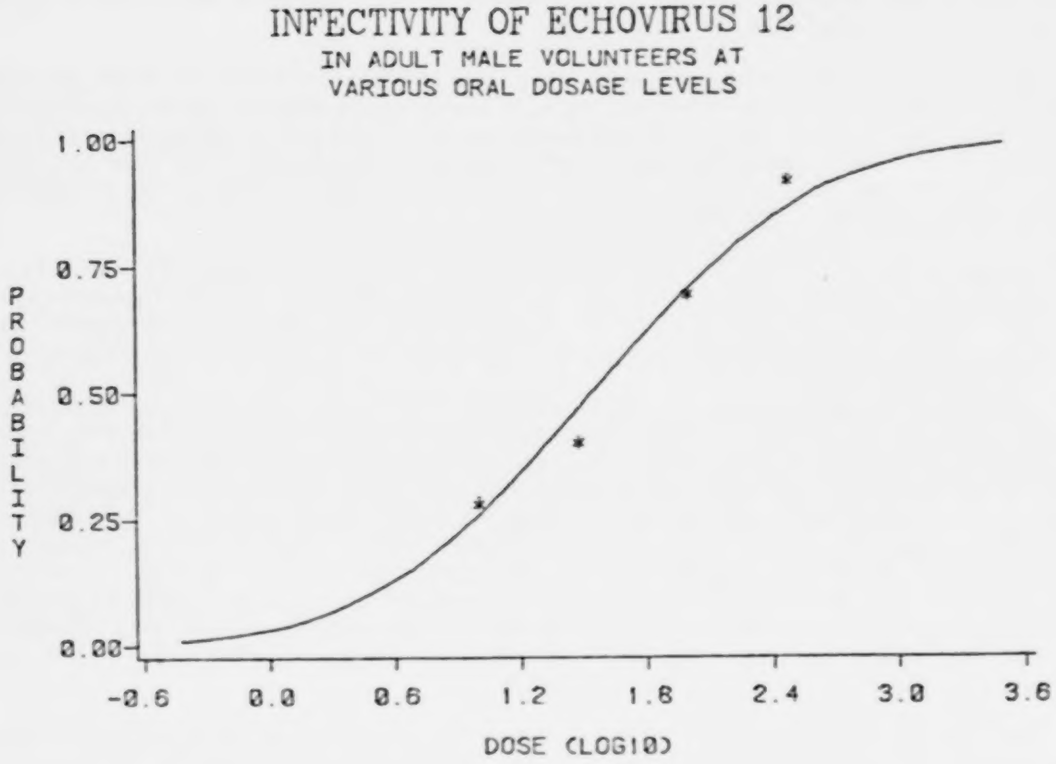

Figure 1. Probit analysis of $\log_{10}$ transformated echovirus 12 updated data of Stefanovic et al. (24). P value associated with Chi square goodness of fit test was 0.6783.

DISCUSSION AND CONCLUSION

Theoretically, all pathogens should be capable of initiating infection with a single organism. The classification, pathogen, itself indicates that the organism possesses the ability to metabolize and/or multiply within the host. With enteric bacteria, the failure of a single organism to colonize may be due to the unique intestinal environment at the time of ingestion. Perhaps diet, a specific normal flora or the "healthy" status of a susceptible host may contribute to a hostile environment for the pathogen preventing survival of the single cell. The ingestion of large numbers may allow the organism selection advantages or the opportunity to compete more effectively, thereby bringing about a favorable environment for colonization.

In this case, host and parasite factors mentioned in the introduction would seem to be major influences on the outcome of exposure. The importance of the health status of the host was shown in a nosocomial outbreak of salmonellosis. The ingestion of as few as 15,000 cells of Salmonella cubana was believed to have initiated disease in patients with altered gastrointestinal function (25). Similarly, DuPont et al. (14) showed that the administration of 2g of sodium bicarbonate 5 minutes before ingestion of an invasive strain of Escherichia coli resulted in severe diarrhea at a dose of 10^6 organisms, 100-fold fewer organisms than required in the absence of the antacid. Such changes in the physiology of the intestinal tract produced by medication and poor health could increase susceptibility to many enteric organisms.

Shigella spp. seem to be unique among the enteric bacteria in their ability to produce disease. Even in compromised hosts, illness is not seen on ingestion of the same number of cells of other bacterial pathogens as on ingestion of Shigella organisms by healthy individuals. These bacteria seem to possess characteristics that can all but insure their survival in the human intestinal tract as indicated by a high percentage of illness on ingestion of as few as 10 cells.

Likewise, amoebic parasites appear to be readily adapted to survival and multiplication in the human intestine. Perhaps the ability of one ingested cyst to initiate infection is limited only by a requirement for physical entrapment in the intestinal villi long enough to excyst and initiate the multiplication process.

Infection by enteric viruses is a completely different process from that of other enteric pathogens. Before a virus can enter a cell and establish infection, specific attachment to the cell surface must take place. The existence of complementary receptor sites on the host cell and the virion surface is the major factor in host specificity to these agents. Apparently only a limited number of cell types exposed in the lumen of the intestinal tract are receptive to virus attachment. Therefore, infection appears to be mainly controlled by the probability of the contact-adsorption process occurring. A host-dependent variable important in the occurrence of this process could be variations in the population size of susceptible cells due to age, health status, etc. Probability of infection would increase with the ingestion of greater numbers of viruses and/or increases in the number of susceptible cells.

The data of Koprowski et al. (19) and Katz and Plotkin (22) showed a high degree of infectivity for poliovirus when the virions were delivered directly to susceptible cells, perhaps lymphoid tissue such as Peyer's patches. Minor's (23) data indicated somewhat larger doses may be required when virus exposure is through more normal routes. The importance to infective dose of direct delivery to the infection site has been clearly demonstrated in studies with common cold viruses. D'Alessio (unpublished data) determined a 50% human infective dose of 0.9 $TCID_{50}$ for a rhinovirus 16 preparation when the virus was placed directly into the posterior nasopharynx. However, as many as 56,000 $TCID_{50}$ were required to infect 1 of 5 subjects when the virus was placed just outside the external nares.

The data of Minor et al. (23) have important practical relevance in that they provide an indication of the actual probability of infection from a natural exposure route, i.e., drinking water. One might assume that the data show that there is a probability of 1:100 that at least one of 20 viral units placed in the oral cavity will survive the tortuous trip through the gastrointestinal tract and make specific attachment to and entry into a susceptible cell of a human infant. The data of Stefanovic et al. (24) are more remarkable in that they indicate that this outcome is more likely with echovirus 12 and that the ingestion of as few as 1 to 2 PFU of this virus could initiate infection in healthy adults.

It should be noted here that a cell-culture-detectable unit of virus is not equivalent to a single virion. Electron microscopy of virus preparations indicates that a number of morphologically indistinct virus particles exists for each infectious unit determined in cell culture. Whether these viruses are also non-infectious for man has not been determined. However, there is no logical reason to assume that cells in culture have reduced susceptibility to viruses compared to those in the intact host. It should also be noted that cell-culture-detectable units of virus may be "stable" clumps of viruses that may be broken up in the host to numerous infectious units of virus. Dose response studies are normally conducted with purified virus preparations that remove cell debris and virus clumps. However, virus probably occurs in nature as clumps of various numbers of virus particles. Obviously, this factor influences the accuracy of exposure values and could result in an underestimation of the dose if destabilization occurred in the host. The virus preparations used to dose the subjects in both EPA-sponsored studies were shown to be relatively free of virus clumps.

The virus data reviewed in this report leave little room for disagreement with the conclusions of the Safe Drinking Water Committee: "there is no valid basis on which a no effect concentration of viral contamination in finished water might be established" (21). This conclusion was reached without the benefit of the echovirus 12 data. It should be mentioned that "no effect" includes asymptomatic infections, a response that is not synonymous with pathogenesis. With wild poliovirus, it has been shown in children that 1,000 infections occur for each paralytic case. Of course, these data do not suggest that this is a typical relationship. The same study indicated that the ratio for adults may be only 75 to 1 (1).

These data indicate that enteric pathogens can cause infection and perhaps disease at levels that could be encountered through environmental exposures to wastewater. Of course, factors other than "infective dose" are important in determining health risk associated with these exposures. Many obviously result in reducing the risk, otherwise a large segment of the population would be constantly experiencing enteric infections and perhaps disease. However, in making judgments on the health hazards in this context, it should be kept in mind that these data were obtained with healthy subjects. Other more susceptible segments of the population must be considered in making risk assessments from exposure to these organisms.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

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MINIMUM INFECTIOUS DOSE: ELMER AKIN

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: Lennette

The basic question that comes to mind is: how do all of these data and findings and observations and experiments relate to the real world. Somehow or other we must look at all of this and recall that the experiments of nature are so extraordinarily complicated that we do not know all the host factors and environmental conditions which determine the susceptibility or resistance of a host to an invading parasite. Even so, it seems to me we have to look at the experimental data presented, data which are important and ostensibly derived from well-planned and well-executed experiments, and ask ourselves how these observations really relate to natural phenomena. We have to give a hard, penetrating look at the role of one or two viral particles in the kind of study mentioned, or at the relationship of a hundred or a thousand salmonellae given to volunteers in a glass of milk, as these relate to outbreak situations which are remarkable events that take place by extraordinary chance under extraordinary circumstances.

There are two things in the environmental science literature which are either misleading or have been misconstrued. The first is the oft-cited New Delhi outbreak I mentioned yesterday, and the other is the work of Plotkin et al. on the susceptibility of prematurely born infants to infection by poliomyelitis virus. This was a highly artificial experiment analogous to those frequently done in a virus laboratory. Thus, one of the participants in this meeting mentioned the use of pathogen-free pigs, which in the real world are hardly normal animals. Many studies in the laboratory utilize infant mice or suckling mice because they are much more highly susceptible to infection than are adult mice, which indeed may be completely resistant. In the case of premature infants the viral inoculum was directly placed, via gavage, into an area of highly susceptible cells in a host ostensibly immunologically compromised to a varying extent. The relevance of any findings from such experiments to events in the real world is questionable.

I am delighted to see that, for the first time, we are beginning to acquire data that will provide dose-response curves, information that we absolutely need if we are ever to estimate scientifically and accurately the infectivity, and thus the public health and medical importance, of viruses in water or in sludge or whatever.

A comment on 50% infectivity and mortality endpoints. These endpoints are statistically determined, and are based on the realization that animals are biologically different one from another so that an endpoint cannot be based upon the use of one guinea pig or one mouse (or one cell culture) but based upon the use of groups of animals. Additionally, if one were ever to obtain a 50 percent endpoint experimentally, thousands of mice or tissue cultures would be required, an obvious impossibility, or at least impracticality. However, by the use of a statistical method such as that of Reed-Muench or Karber, a 50 percent infectivity or lethality endpoint can be determined by the use of relatively small numbers of animals. With reference to cell cultures, theoretically one single particle gives rise to one plaque-forming unit, although we really do not know whether a single viral particle is involved or whether it is a clump of five or six or more -- all we know is that a single lesion has been produced and theoretically this is due to a single particle. The occurrence of viral aggregates should be kept in mind when referring to plaque-forming units or infectivity or lethality doses.

A: Akin

I would like to respond to some of your comments. You know, Dr. Lennette, I am very sensitive to the points you raise because our lab has probably been the group within the Agency (EPA) that has been more critical of the literature in this area. That is why I really looked for studies in the literature for this review that seemed to have a practical significance; Katz and Plotkin's data were included because they are cited so often. The two studies funded by the Agency utilized exposure levels pretty close to practical exposure levels. At least the virus was placed in water and introduced into the oral cavity. The University of Wisconsin study did find a higher dose for infection than did Katz and Plotkin, but sill a relatively low dose was found to produce infection in half of the exposed subjects -- about 70 $TCID_{50}$'s. The Echo 12 data were a shock to me. The concept that a single virus particle could be placed in the mouth and somehow end up in the intestinal tract and infect cells that are susceptible after such a tortuous route is pretty remarkable. I feel that the controls that you have just mentioned were in place in that study. The investigators had a good handle on the dosage and the virus was purified. I don't know if it was totally mono-dispersed, but viruses don't occur in nature in single particles either. If we get a dose in drinking water it is probably not going to be a single particle/ it is probably going to be a clump of some unknown number of particles. In the paper I have expanded a little more on some of these subjects than I have here. I mentioned some of Donn D'Alessio's data that at the time of my report preparation were unpublished. I don't know if they have been published since but they related to the work he did with rhinovirus 16 in which he determined the dosage when the virus was placed in the nasopharynx versus exposure just outside the nares. A considerable difference in infective dose values was obtained.

Q: __________

Well, with the experience with viruses involved here I think it is a lot broader than these kinds of feeding studies, and I think those that have done human volunteer studies with viruses become aware quite quickly that one tissue culture infectious dose will infect people if placed directly into the nasal pharynx but if you put it anywhere else you need a hell of a lot more virus. Placing it on the tongue increases the dosage required for infection many fold. That was, I think what we were seeing with the Plotkin data as giving an artificial picture and this is why we thought it was important to feed the dose orally to get maybe a truer picture of what might be happening. But group after group have found very small doses of virus will infect humans if placed directly on the susceptible cell population.

Q: Lennette

Just one more comment. Since I for a long time was a lone voice in the wilderness, I'm glad to see I'm getting some support. One of the things I object to is the direct translation of tissue culture infectivity data to man. For example, the statement was made here somewhere that anything which is infectious to tissue culture cells ergo is infective for man. This does not necessarily follow. Nor can one compare what occurs in a prematurely-born infant, which may take weeks and maybe a hundred thousand dollars to get up to the normal state, with what actually happens in man. Man isn't a big, helpless hunk of protoplasm lying passively and unprotected on the earth. He has natural resistance, he has immunologic defense mechanisms which go into action as soon as he is confronted with a parasite. The tissue culture cell, that poor thing, however, is lying there absolutely naked with nothing to help it. You can't equate the two.

PUBLIC HEALTH RISK EVALUATION OF WASTEWATER DISPOSAL ALTERNATIVES

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ABSTRACT

The public health risk associated with wastewater disposal alternatives is difficult to establish. A probability matrix method of estimating such risk is presented. An example of the application of the method is given in which the relative health risk of alternative on-site waste disposal systems is estimated.

INTRODUCTION

One of the most important aspects in considering the environmental impact of wastewater disposal and wastewater reuse is that of the effect of such activity upon the public health. Making such a judgment requires some estimate of the risk involved. Risk is not an unfamiliar concept as all of us are constantly subject to an array of risks during our daily lives. However, quantitating these risks is frequently considered impractical because of lack of adequate data.

The term risk as used in this paper is defined as a compound measure describing the probability of an adverse event occurring and the severity in such an advent. Risk assessment involves the process of making estimates that particular adverse events will occur in a given time period; while risk evaluation involves a social value judgement as to what level of risk is acceptable.

Kates (1) has defined three methods used in estimating risk: revelation, intuition and extrapolation. Revelation and intuition, though frequently applied, are generally not accepted as scientific because of their reliance on conjecture and implicit judgment. The last method, extrapolation, involves the extending or projection of known information about a specific event in order to estimate some other event. Moreau (2) has further defined extrapolation to include the following methods: 1) standard statistical methodology; 2) causal models based on known phenominological relationships; and 3) formal methods of encoding expert opinion in the form of judgmental probabilities. Environmental data required by the first two methods of risk assessment frequently do not exist or are not readily available. In real situations, however, decisions by a responsible agency must be made. This paper deals with an application of the third method cited above to the risk assessment of waste disposal alternatives.

The public health implications of various wastewater treatment activities have generally been described in terms of the variety of chemical and biological agents that might be present. In some cases, direct or indirect measure of the concentration of these agents is available. As a rule, implicit judgments of risk are made from such information.

Having been involved in a number of implicit judgment of risk excercises (with the end product expressed as the situation is good or it is bad) we were interested in exploring a more explicit judgment technique. The method adopted was a probability matrix technique. With this method, effective utilization is made of the expertise of public health specialists in arriving at explicit judgments regarding the relative health risk in the application of a given water reclaimation process.

THE PROBABILITY MATRIX TECHNIQUE

The probability matrix technique (PMT) risk assessment method requires the input of two groups of health experts. The first group delineates health problems which may be associated with a given process and estimates the probability of their occurrence. The role of the second group is to judge the severity of the problems delineated by the first group. Members of the first group are instructed to make judgments on the probability of problem occurrence and to place a value on the probability using a linear scale of zero to one. In such a scale, values less than 0.5 indicate that the occurrence of a problem is less likely than its non-occurrence.

A public health problem is defined as a situation in which the incidence of disease or level of annoyance increases above background to the degree that action by a health agency would be indicated.

Members of the second group are directed to make judgments concerning the severity of the problems named by the first group. Relative severity is ranked from zero to 100, with 100 being the most severe and zero the least. If necessary, the same value can be associated with more than one problem. In this instance, the severity of a problem must be considered in terms of the impact of a given disease agent or environmental factor on the health of an individual who has contracted said disease or disability. Relative judgments as to the severity of a problem are in comparison to the other problems being considered and not to some absolute level of severity. The severity judgments are medical opinions based on collective knowledge of the life expectancy, degree of disability, and effectiveness of treatment of the public health problems considered. The membership of the first group would include experts in bacteriology, virology, parasitology, entomology, sanitary engineering and similar professions. The second group of experts would most likely be composed of selected physicians.

From the judgments made by the two groups of experts, an estimate of the magnitude of the risk of a health problem related to any given wastewater disposal process can be determined. The calculation of the magnitude of the risk is as follows:

$$r_i = \frac{\sum_{j=1}^{n} P_{ij} S_j}{\sum_{j=1}^{n} S_j} \qquad (1)$$

Where r_i is the magnitude of risk (0-1); P_{ij} is the subjective probability of a problem, j will occur under condition $i(0 \leq P_{ij} \leq 1)$; S_j is the relative severity of problem $j(0 \leq S_j \leq 100)$; n is the number of public health problems (j) being evaluated.

An example of the use of this method is shown in Tables I and II. In 1973 (3), we were asked to evaluate the public health implications of the proposed land disposal of reclaimed wastewater at a number of sites in California. We used the probability matrix technique employing two groups of experts as previously described. Twenty-three potential health problems were identified and are listed in the tables. There were a total of eight proposed sites about which appropriate information was supplied to the expert group. Table I illustrates the calculation of r for one of the sites and Table II is a complete assessment for all sites. The outcome suggested that there were three groups of sites. The first, Site 4 was considered the least hazardous; the second group (Sites 28, 42, 43 and 21) were next and Sites 27, 5 and 18 were third. At all sites, the risk estimate was less than 0.5 which incicate that, based upon expert judgment, there was less chance of disease occurring than not occurring. This method also makes the judgment of the experts explicit and gives the reviewer a chance to challenge and the expert a chance to defend his P_{ij} values.

More recently, we have had an opportunity to apply the probability matrix approach with some modifications to the question of the public health risk involved in the use of alternative individual waste disposal systems. The following is a discription of our approach to this risk evaluation problem.

As the population of the United States increases, the demand for new housing grows accordingly. In many cases, the resulting new development is occurring in proximity to existing urban areas and within the service boundaries of areas served by municipal sewerages agencies. But, in an increasing number of instances, development is being proposed in outlying and rural areas which cannot easily be served by existing agencies. A decade ago, the septic tank system had come to be accepted as the conventional solution to wastewater treatment and disposal.

During the past several years, this acceptance of the septic tank system has begun to be seriously eroded. The reason is that in many areas, soil and other site conditions make septic tank systems unsuitable. As a result, local health and water pollution control agencies are manifesting a growing determination to mandate against the use of septic tanks in such areas. A consequence of the prohibition is that unless a satisfactory substitute for the septic tank can be found, land in those areas is not allowed to be developed. Attempts to find a satisfactory substitute have spawned an array of alternatives, some of which are shown in Figure 1 and described in Appendix I. Chances are that one or another of the substitutes can provide the safe treatment and disposal that would permit lands to be developed on which septic tank treatment and disposal would not be suitable. A serious obstacle to the utilization of the substitutes is, in most cases, a lack of defined review criteria on which a regulatory agency can base its decision to approve or to disapprove.

An evaluation of the health risks posed by the use of on-site waste disposal systems requires knowledge concerning: 1) the presence of agents that cause disease; 2) the dose response characteristics of the agents involved; and, 3) the manner in which the agent comes into contact with susceptable individuals. Unfortunately, in many instances, data on one or more of the preceding items are not available.

TABLE 1

PROBABILITY MATRIX SAMPLE CALCULATION - SITE 5

Sample Calculation for Site i = 5

j	Medical Problem	S_j	P_{5j}	S_jP_{5j}
1	Rabies	100	.25	25.00
2	Enteric virus polio	100	.08	8.00
3	Plague	87	.23	20.01
4	Coccidioidomycosis (blacks)	83	.98	81.34
5	Infectious hepatitis	83	.09	7.47
6	Encephalitis	52	.98	50.96
7	Typhoid	49	.08	3.92
8	Heavy metal effects: lead and zinc/cadmium	47	.21	9.87
9	Murine typhus fever	42	.04	1.68
10	Other salmonella	39	.08	3.12
11	Parasitical: E. hystolytica	33	.02	.66
12	Coccidioidomycosis (whites)	24	.98	23.52
13	Shigella	21	.08	1.68
14	Nitrates effect	20	.00	.00
15	Malaria	17	.20	3.40
16	Tularemia	15	.75	11.25
17	Leptospirosis	14	.47	6.58
18	Nuisance: pasture mosquitoes	7	.99	6.93
19	Enteric virus: adeno	4	.08	.32
20	Nuisance: biting flies	4	.99	3.96
21	Enteric virus: echo	0	.08	.00
22	Nuisance: rodents	0	.73	.00
23	Nuisance: odors	0	.00	.00
	TOTAL	841		269.67

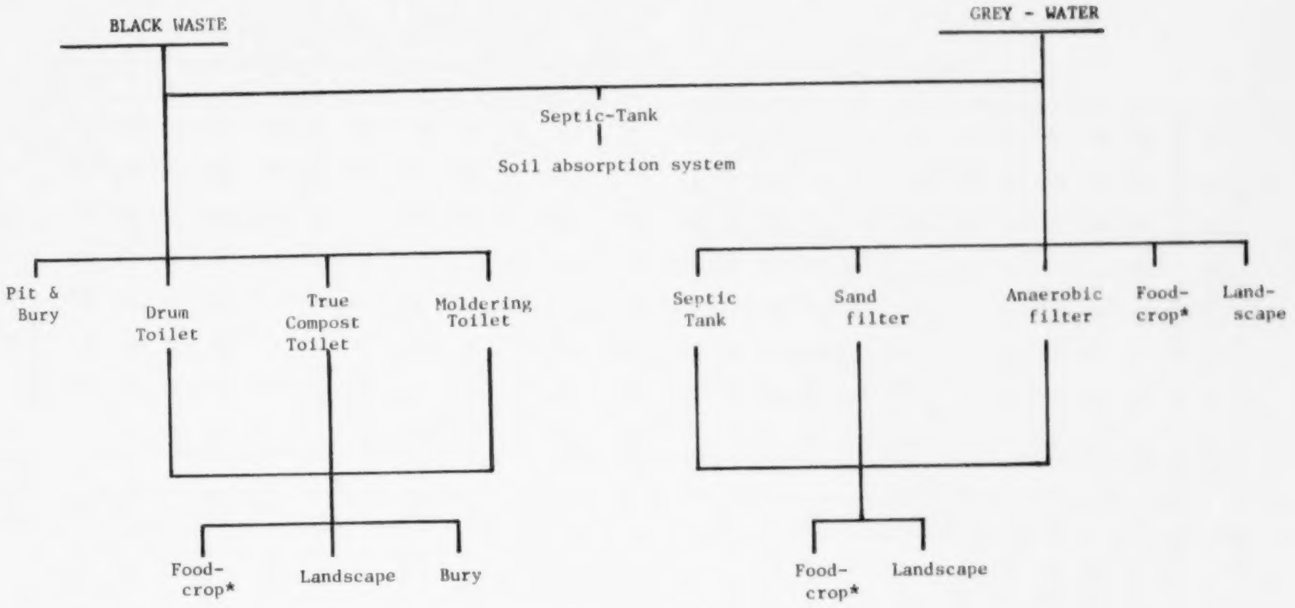
$$r_5 = \Sigma S_j P_{5j} / \Sigma S_j = 269.67/841 = .32$$

TABLE 2

ASSESSMENT OF EXPECTED MAGNITUDE OF PUBLIC HEALTH PROBLEMS BY SITE

Problem	rabies	enteric virus polio	plague	coccidioidomycosis (blacks)	infectious hepatitis	encephalitis	typhoid	heavy metal effects: lead and zinc/cadmium	murine typhus fever	other solmonella	parasitical: e. hystolytica	coccidioidomycosis (whites)	shigella	nitrates effect	malaria	tularemia	leptospirosis	nuisance: pasture mosquitoes	enteric virus: adeno	nuisance: biting flies	enteric virus: echo	nuisance: rodents	nuisance: odors	
S_j	100	100	87	83	83	52	49	47	42	39	33	24	21	20	17	15	14	7	4	4	0	0	0	
Site	P_{ij}																							r_i
4	.03	.04	.04	.00	.04	.26	.04	.00	.04	.04	.02	.00	.05	.00	.02	.04	.07	.48	.04	.48	.04	.03	.00	.05
5	.25	.08	.23	.98	.09	.98	.08	.21	.04	.08	.02	.98	.08	.00	.20	.75	.47	.00	.08	.99	.08	.73	.00	.32
18	.74	.85	.50	.00	.36	.50	.34	.26	.04	.37	.02	.00	.20	.26	.02	.20	.23	.99	.35	.99	.35	.47	.00	.34
21	.50	.04	.50	.00	.03	.74	.03	.04	.04	.04	.02	.00	.04	.18	.02	.75	.45	.99	.04	.99	.04	.48	.00	.21
27	.50	.03	.49	.84	.03	.50	.03	.11	.04	.03	.02	.84	.03	.05	.02	.50	.45	.99	.03	.99	.03	.75	.00	.30
28	.51	.03	.48	.00	.03	.21	.03	.26	.04	.03	.02	.00	.03	.19	.02	.03	.05	.99	.03	.99	.03	.48	.00	.17
42	.20	.03	.20	.16	.04	.74	.03	.20	.04	.03	.02	.16	.03	.13	.02	.48	.05	.99	.03	.99	.03	.48	.00	.16
43	.19	.04	.20	.16	.04	.95	.03	.26	.04	.04	.02	.16	.04	.26	.20	.71	.20	.71	.20	.99	.04	.99	.00	.19

Note: S_j = severity of problem (medical); P_{ij} = probability of occurrence of problem;
r_i = magnitude of public health problem.


BLACK WASTE
GREY - WATER
Septic-Tank
Soil absorption system
Pit & Bury
Drum Toilet
True Compost Toilet
Moldering Toilet
Septic Tank
Sand filter
Anaerobic filter
Food-crop*
Land-scape
Food-crop*
Landscape
Bury
Food-crop*
Landscape

* Rooted or leafy crops intended for human consumption

Figure 1. On-site waste disposal systems.

The disease agents involved may be classified as infectious and non-infectious. The infectious agents of most importance in considering alternative disposal systems are those of intestinal origin. The would comprise three main groups, namely, bacteria, eucaryotic and multicellular parasites and viruses. Zoonotic diseases may also be involved as a result of the mismanagement of wastewater discharge onto the land, a major characteristic of many on-site systems. The non-infectious disease agents can be further divided into two classes: toxic chemicals and nuisance conditions. A major toxic chemical associated with on-site systems is nitrate. Relative to nusiance conditions is the on-site systems potential for encouraging the breeding of insects and the production of odors.

Information on human dose response to infectious and non-infectious disease agents is limited. Some information is available on this relationship between man and certain bacterial and parasitic diseases (4). As might be expected, the dose response varies from one agent to another. In the case of bacterial enteric disease, it usually takes a large number of organisms to elicit a significant response in challenged individuals (as few as 100 Shigella sp. to 1×10^8 cholera vibrios to to produce 25 to 75 percent response in man). In the case of parasitical diseases such as Giardiasis, the dose required to produce infection may range from 10 to 1×10^6 cysts per individual exposed. At the time of this writing, there is a great deficiency in dose response data involving enteric viruses.

In order to contract a disease, the susceptible host must come into contact with an effective dose. The contact may be direct or indirect. An example of an indirect contact is that resulting from the utilization of grey water (raw or treated) for the irrigation of food plants. Another is the contact made possible through the deposition of treated or untreated fecal matter on soil, thereby providing the potential for the contamination of food with pathogens. An expample of direct contact is drinking and cooking with water that has been contaminated with leachate from a poorly sited septic tank system. Obviously, the more direct the contact, the more certain it is that an effective dose will reach the host.

Another aspect of dose contact involves the density of the exposed population. Here, an important consideration is the number of individual on-site systems that can function in a given locale without increasing the potential for disease transmission to an unduly high level, or without the development of unacceptable aesthetic conditions.

The assessment method employed two steps in arriving at estimates of the public health risk of the systems involved. The process previously described was used (see Equation 1). The r_i value was determined for each type of alternative system; however, a second step was included in which a series of r values were determined for the system under various conditions of climate, depth of ground water, soil percolation times, depth to bedrock , land slope and population density (see Appendix II for definitions). This second step combines the incremental risk estimates:

$$R = r_1 \times r_2 \times r_3 \ldots r_n \qquad (2)$$

where R is the probability of a public health problem over the life of a system.

The key assumption made in using these risk equations is that all risks act independently. In actuality, such an assumption may not be true; however, if the problem and environmental condition categories are judiciously taken and grouped, the assumption may become more acceptable. A consequence of the independence assumption is that the net probabilities can be obtained rather easily. The process requires the development of a matrix which produces the estimated r_i value for each type of system or system combinations under the selected conditions. An example of such a matrix is shown in Figure 2. The twenty health problems to be considered are listed as a heading of this figure.

The matrix values of probability of a problem occurring (P_{ij}) for each type of alternative disposal system was determined by a group of professionals knowledgeable in these matters. In this instance, the determinations were made as part of the activity of the California State Department of Health Services, Rural Wastewater Disposal Alternatives Project funded by the U.S.E.P.A. As part of this larger study, an outside committee of professionals was convened. This group was composed of seven members representing the fields of microbial infectious diseases, parasitology, medical entomology, public health sanitation, sanitary engineering, and solid waste management. The severity of problem estimates (S_j) were developed by a group of five public health physicians. Their first task was to develop the list of problems (j).

Estimates of the probability of the occurrence of a problem were obtained using the Delphi technique. This technique is a method of applying the collective knowledge or abilities of a diverse group of experts to the task of quantifying variables that either are intangible or are shrouded in uncertainty. The Delphi technique can be used to develop estimates of P_{ij}, i.e., the probability of a problem occurring. The term "expert", as defined by the developers of the technique (Rand Corporation), generally implies a highly educated and experienced specialist.

The Delphi technique involves the use of a series of questionnaires designed to produce group consensus; and to eliminate face-to-face confrontation as is experienced in panels or committees. The key characteristics of the Delphi approach are anonymity, controlled feedback, and statistical group response. Anonymity is attained through the use of questionnaires. In this way, the effect of an undue influence of dominant panel members upon the responses of other panel members is avoided. Controlled feedback is attained by conducting the exercise in a sequence of rounds, between each of which a summary of the results of the previous round is communicated to the participants. The effect is a reduction in "noise". Use of the statistical group response reduces group pressure for conformity; and assures that the opinion of every member is represented in the final response.

During the first round, the committee members were requested to provide estimates of P_{ij} for only those combinations of problems and conditions for which they felt qualified to estimate values. During the second round a statistical summary of the first round was returned to each participant and if any changes in their original estimate were desired, such changes could be made. The majority of the members believed their estimates and made no changes. When changes were made by an individual, they were prompted by a desire either to adjust to the group mean, or by the feeling that other group members knew more about the problem. The mean values of P_{ij} were than recalulated.

The severity values were solicited from the five public health physicians. The values were developed on a linear scale of zero to 100 as previously described. The derived mean values of the public health problem severity (S_j) are shown in Table 3.

Following Equation 1, the incremental risk values were calculated using the

Severity (S_j)																					r_i
PROBLEM j CONDITION i	Enteric Viruses	Polioviruses	infectious hepatitus	encephalitis	Typhoid	Salmonella	Shigella	Ascaris	hookworm	Giardia	Entamoeba histolytica	cistodes	animal parasites	beetles	rodents	flies and gnats	mosquitoes	venemous arthropods	odors	nitrates	risk
Climate: Mountain																					
Desert																					
Coastal																					
Ground-water: < 3 feet																					
3 - 5 feet																					
> 5 feet																					
SOIL PERCOLATION: faster than 45 $\frac{min}{in}$																					
45 - 60																					
slower than 60																					
DEPTH TO BEDROCK: < 4 feet																					
> 4 feet																					
TOPOGRAPHY SLOPE: 0 - 10%																					
11 - 30%																					
> 30%																					
DENSITY: Low A																					
B																					
Medium A																					
B																					
High A																					
B																					

Figure 2. Example of a probabilty matrix to estimate the r_i values for a given disposal system under a variety of conditions (see appendix II for condition definitions)

mean of P_{ij} values obtained in the second Delphi round and the mean S_j values of Table 3. These incremental (r_i) values for all the disposal systems considered and for each condition are shown in Table 4. Using the results shown in Table 4 and following Equation 2, an estimate of the total system risk can be obtained. The results of the total risk (R) for a number of systems under similar conditions is shown in Table 5.

Estimates of the public health risk of combined black waste and grey-water disposal systems are shown in Table 6. These estimates were made by calculating the composite event of the probability of a problem occurring when both systems are operative.

The result of this exercise indicates that all dry toilets which utilize waste disposal through burying of the solids are estimated to present the same degree of risk and said risk is the same as that encountered with the septic-tank soil absorption system. Disposal of wastes from the moldering and drum toilets to food crops is estimated to be 145 times the risk value of disposal by burying, while disposal from a true composting toilet on to a food crop is 24 times the risk value of burying the solids. The use of moldering and drum toilets with solids disposal to food crops is estimated to be six times the risk value of the use of the true compost toilet with disposal to food crops. Raw grey-water disposed onto food crops have an estimated risk value four times that of disposal onto landscape and 70 times that of the septic-tank system. An estimate of the public health risk of combined black-water from dry toilets and grey-water systems (see Table 6) indicates a 17 to 215 fold greater risk than that attending the use of the septic-tank system.

This health risk assessment technique brought out several factors that were important in affecting risk judgment. These factors included the disposal system conditions, end product disposal, system maintenance and disease morbidity in the associated population.

The disposal system conditions of climate and population density play a predominant role in the determination of estimated risk values. Climate was considered a major variable since conditions of temperature, rainfall and sunlight have a significant impact upon the survival and transport of disease agents that might be present in black waste or grey-water. A desert climate was associated with the least risk, while mountains and coastal climates were felt to constitute a somewhat greater hazard. From the list of incremental risks shown in Table 4, it can be seen that the risk estimate tended to double when the system use conditions changed from low to high population density.

The degree of risk, as shown by these estimates, is heavily dependent upon the final use (disposal) of the black waste and grey-water. As one might suspect, risk estimates increased in the following order: bury < landscape < food crop. This obviously reflects the experts' judgment concerning the mode of dose-contact.

In making health risk estimates, the group of experts assumed that the on-site systems would be properly maintained. Thus, this factor was not a variable included as a system condition. The group felt that the impact of poor maintenance would have a minor effect on public health risk values as long as systems were used in lightly populated areas. This means that improper maintenance may increase the risk to the individual involved in such maintenance activity, but the development of a public health problem as defined would most likely not occur.

TABLE 3

MEAN SEVERITY VALUES OF PUBLIC HEALTH PROBLEM

PROBLEM	MEAN SEVERITY*
Enteric Viruses	58
Polioviruses	89
infectious hepatitis	79
encephalitis	100
Typhoid	72
Salmonella	47
Shigella	53
Astaris	25
hookworm	41
Giardia	36
Entamoeba histolytica	59
cestodes	36
animal parasites	24
beetles	0
rodents	40
flies and gnats	37
mosquitoes	47
venemous arthropods	66
odors	9
nitrates	44

*Relative Linear Scale of zero to 100.

TABLE 4

INCREMENTAL RISK VALUES (r_i) FOR EACH DISPOSAL SYSTEM UNDER A VARIETY OF CONDITIONS

CONDITION i		PIT AND BURY	DRUM TOILET TO FOOD CROP	DRUM TOILET TO LANDSCAPE	DRUM TOILET AND BURY	TRUE COMPOST TO FOOD CROP	TRUE COMPOST TO LANDSCAPE	TRUE COMPOST AND BURY	MOLDERING TOILET TO FOOD CROP	MOLDERING TOILET TO LANDSCAPE	MOLDERING TOILET AND BURY	SEPTIC TANK-SOIL ABSORPTION SYSTEM	GREY-WATER TO SEPTIC TANK TO LAND	GREY-WATER TO SEPTIC TANK TO FOOD CROP	GREY-WATER TO SAND FILTER TO FOOD CROP	GREY-WATER TO SAND FILTER TO LANDSCAPE	GREY-WATER TO ANAEROBIC FILTER TO F.C.	GREY-WATER TO ANAEROBIC FILTER TO L.	GREY-WATER TO FOOD CROP	GREY-WATER TO LANDSCAPE
Climate: Mountain		.3	.4	.3	.2	.4	.2	.1	.4	.3	.2	.2	.2	.2	.3	.2	.3	.2	.3	.2
Desert		.3	.4	.3	.2	.3	.1	.1	.3	.2	.1	.1	.1	.2	.2	.1	.2	.2	.3	.2
Coastal		.3	.4	.3	.2	.4	.2	.1	.4	.3	.2	.2	.2	.3	.3	.2	.3	.2	.3	.2
Ground-water:* < 3 feet		.4	.3	.2	.3	.2	.2	.2	.3	.2	.2	.3	.2	.3	.3	.2	.2	.2	.3	.2
3 - 5 feet		.3	.2	.2	.2	.2	.1	.2	.2	.2	.2	.2	.2	.2	.2	.2	.2	.2	.2	.2
> 3 feet		.2	.2	.1	.1	.1	.1	.1	.2	.1	.1	.1	.1	.2	.2	.1	.2	.1	.2	.1
SOIL PERCOLATION: faster than 45 $\frac{min}{in}$		.3	.2	.2	.2	.2	.2	.1	.2	.2	.2	.2	.2	.2	.2	.1	.2	.2	.2	.2
45 - 60		.2	.2	.2	.2	.2	.1	.1	.2	.2	.1	.1	.2	.2	.2	.2	.2	.2	.2	.2
slower than 60		.2	.2	.2	.2	.2	.1	.1	.2	.2	.1	.2	.2	.2	.2	.2	.2	.2	.3	.2
DEPTH TO BEDROCK: < 4 feet		.3	.3	.2	.2	.2	.2	.2	.3	.2	.2	.2	.2	.2	.2	.2	.2	.2	.3	.2
> 4 feet		.2	.2	.2	.1	.2	.1	.1	.2	.2	.1	.1	.1	.2	.2	.1	.2	.1	.2	.2
TOPOGRAPHY SLOPE: 0 - 10%		.2	.3	.2	.2	.2	.1	.1	.2	.2	.2	.1	.2	.2	.2	.2	.2	.2	.2	.2
11 - 30%		.2	.3	.2	.1	.2	.1	.1	.3	.2	.1	.2	.2	.1	.2	.2	.2	.2	.2	.2
> 30%		.3	.3	.2	.2	.2	.2	.1	.3	.2	.2	.3	.2	.1	.2	.2	.2	.2	.2	.2
DENSITY: Low	A	.2	.3	.2	.1	.2	.1	.1	.3	.2	.1	.1	.1	.2	.2	.1	.2	.1	.2	.2
	B	.2	.3	.2	.1	.2	.1	.1	.3	.2	.1	.1	.1	.2	.2	.1	.2	.1	.2	.2
Medium	A	.3	.3	.3	.1	.2	.2	.1	.3	.2	.1	.1	.2	.2	.2	.2	.2	.2	.3	.2
	B	.3	.4	.3	.2	.3	.2	.1	.3	.3	.2	.2	.2	.2	.2	.2	.2	.2	.3	.2
High	A	.3	.4	.3	.2	.3	.2	.2	.4	.3	.2	.2	.2	.3	.3	.2	.3	.3	.3	.2
	B	.4	.4	.4	.3	.3	.3	.2	.4	.3	.3	.2	.2	.3	.3	.2	.3	.3	.3	.3

* depth from ground surface; assume potable water.

TABLE 5

INCREMENTAL AND TOTAL SYSTEM RISK VALUES FOR DISPOSAL SYSTEMS UNDER SAME CONDITIONS

CONDITION / SYSTEM	COASTAL	GROUND WATER > 5 feet	SOIL PERCOLATION 45-60 $\frac{\text{min}}{\text{in}}$	DEPTH TO BEDROCK: > 4 feet	SLOPE: 11-30%	DENSITY: Medium (A)	Total System Risk R Probability of a Public Health Problem/System life
SEPTIC TANK SOIL ABSORPTION SYSTEM	.2	.1	.1	.1	.1	.1	2×10^{-6}
MOLDERING TOILET TO LANDSCAPE	.3	.1	.2	.2	.2	.2	4.8×10^{-5}
MOLDERING TOILET TO FOOD CROP	.4	.2	.2	.2	.3	.3	2.9×10^{-4}
DRUM TOILET TO LANDSCAPE	.3	.1	.2	.2	.2	.3	7.2×10^{-5}
DRUM TOILET TO FOOD CROP	.4	.2	.2	.2	.3	.3	2.9×10^{-4}
TRUE COMPOST TO LANDSCAPE	.2	.1	.1	.1	.1	.2	4×10^{-6}
TRUE COMPOST TO FOOD CROP	.3	.3	.2	.2	.2	.2	4.8×10^{-5}
GREY-WATER TO LANDSCAPE	.2	.1	.2	.2	.2	.3	3.2×10^{-5}
GREY-WATER TO FOOD CROP	.3	.2	.2	.2	.2	.3	1.4×10^{-4}
PIT PRIVY	.3	.2	.2	.2	.2	.3	1.4×10^{-4}
True Compost, Moldering Toilet & Drum Toilet to Bury	.2	.1	.1	.1	.1	.1	2×10^{-6}

Density: medium (A) is defined as less than 20 systems, 1-5 acres per system, normal population distribution, and 2.5 persons per system

TABLE 6

ESTIMATE OF HEALTH RISK IN THE COMBINED DISPOSAL OF BLACK WASTE AND GREY-WATER*

Black Waste System	Combined Risk Estimate	
	Raw Grey-Water to Landscape	Raw Grey-Water to Food Crop
Septic Tank-Soil Absorption System	2×10^{-6}	
True Compost, Moldering and Drum Toilet to Bury	3.4×10^{-5}	1.4×10^{-4}
True Compost to Landscape	3.6×10^{-5}	1.4×10^{-4}
Moldering Toilet to Landscape	8×10^{-5}	1.9×10^{-4}
True Compost to Food Crop	8×10^{-5}	1.9×10^{-4}
Drum Toilet to Landscape	1×10^{-4}	2.1×10^{-4}
Pit Privy	1.7×10^{-4}	2.8×10^{-4}
Moldering Toilet to Food Crop	3.2×10^{-4}	4.3×10^{-4}
Drum Toilet to Food Crop	3.2×10^{-4}	4.3×10^{-4}

* Composite event of probability of a public health problem occurring with the black waste system or with the grey-water system; (i.e., $p(A \text{ or } B) = p(A) + p(B) - p(AB)$ where A and B represent the black and grey-water system respectively.

The health risks associated with on-site waste treatment and disposal systems will vary with the disease morbidity in associated populations. Reported enteric infectious disease morbidity in the United States is generally low and, consequently, agent doses are low and dose contact limited. Thus, documented disease incidents associated with on-site systems are relatively rare events. Conditions outside the United States may be very different, especially in less developed and highly populated countries. Therefore, the values generated by this present exercise would not universally apply.

SUMMARY AND CONCLUSIONS

Frequently, judgment concerning the health risk associated with wastewater disposal alternatives must be made with an insuffiency of data. The risks assessment approach described herein is highly conducive to the formation of insights into the public health implications of disposal alternatives. The assessment method renders explicit the judgment of experts by recording the information used by them and the risk probability value assigned to a particular activity for the decision-maker's review. The methodology used is certainly subject to improvement. The risk values developed are, by no means, absolute probabilities, but can serve as a useful tool in community planning and governmental review. The exercise tends to place potential health problems into a proper perspective and give one a sense of their magnitude.

ACKNOWLEDGMENTS

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APPENDIX I. ON-SITE WASTE TREATMENT AND DISPOSAL SYSTEMS DESCRIPTION

BLACK WASTE SYSTEMS

Pit Privy

A pit privy consists of a hand-dug or bored pit over which a shelter is placed for privacy. After the pit is filled, a new hole is dug and the structure is moved to the new site. Filled pits are covered with two feet or more of soil.

Moldering Toilet

"Moldering toilet" is a term loosely applied to a broad group of self-contained (vault-type) toilet systems, in which the addition of organic matter of plant origin to human excreta is a common characteristic. The group is divided into two principal subdivisions on the basis of whether or not aeration is accomplished passively or actively. Active aeration implies either forced aeration by mechanical means, or the introduction of air by turning or equivalent mixing. A grey area in terms of definitions arises here. If the aeration is truly effective, the particular system may be validly designated as a "true compost toilet." The beneficial features popularly associated with composting apply only to actively and effectively aerated systems.

True Compost Toilet

The designation "true compost toilet" is given to a dry toilet in which provision is made for the active control of the decomposition of a raw human excreta into a sanitary soil amendment by exposure to indigenous microorganisms, heat, time, and sufficient oxygen in the presence of adequate moisture. The control involves the provision of effective aeration, usually through "turning" the material. Effective aeration is indicated by: 1) rise in temperature of the composting mass, provided the mass is sufficiently large to permit self-insulation (about one cubic yard), and 2) the absence of foul odors in the immediate vicinity of the composting mass. The final product from a compost toilet is disposed of through burial, and filling tilling as a soil amendment on landscape.

A factor common to the drum toilet, the moldering toilet, and the true compost toilet is the need for handling when disposing of the final product.

GREY-WATER SYSTEMS

Grey-Water Definition

The term "grey-water" is given to wastewater generated from the nontoilet plumbing fixtures in a residence, i.e., discharge from sinks, basins, bathtub or shower, etc. Grey-water may be used as irrigation water for edible food crops or for ornamentals and lawns.

Sand Filters

The term "sand filters" is applied to a treatment system that consists essentially of a layer of sand provided with an underdrain system but lacking a backwash system. The depth of the sand layer ranges up to 42 inches. Grey-water is applied generally at a rate of about six to eight gal/ft^2/day. The filter is used until the wastewater rises to the top of the filter wall, after which it is removed from the operation. It is then drained, permitted to dry, and finally is cleaned manually to remove the filtered solids. Sand that is lost during the cleaning must be replaced. Sand filters also may be operated on an intermittent basis to allow for the aerobic digestion of solids. The latter method results in a reduction in the required frequency of maintenance.

Septic Tank

The "septic tank: may be described as a watertight, covered receptacle designed and constructed: 1) to receive the discharge of sewage from a building sewer, 2) to separate solids from the sewage, 3) to digest the organic fraction of the solids through a period of detention, and 4) to allow the clarified liquids to be discharged for final disposal.

Soil Absorption System

A soil absorption system is one in which the soil is utilized for the subsequent absorption of the treated sewage. The most common type of soil system consists of trenches lined with perforated pipe, through which treated sewage seeps into the soil system. The pipes are placed in gravel and covered with earth.

Anaerobic Filter

An "anaerobic filter" is essentially a column made up of a solid medium. The medium usually is in the form of 1 1/2 to 2-inch rocks. Anaerobic bacteria grow and are retained on the surfaces of the rocks. As the wastewaters pass through the column, they come into contact with the anaerobic bacteria. Because the bacteria are retained on the media and are not washed off with the effluent, the mean cell residence time can be on the order of 100 days.

APPENDIX II. CONDITION DEFINITIONS

(1) CLIMATE

Mountain: (includes Northwestern Coastal Mountains) elevation over 2,500 feet; several areas receive over 60 inches of rain per year, with some receiving 70-110 inches per year; most areas receive 35-40 inches of rain per year; January average temperature 47°F; August average temperature 74°F; fog is rare; temperatures often exceed 100°F in July and August; above 3,000 feet the mountains have a forest cover with a mixture of several species of pine and other trees and many shrubs.

Desert: January average temperatures range between 46-50°F; July average temperatures range between 93-116°F; thunderstorms occur during the summer; rainfall averages around 1-10 inches per year.

Coastal (lowlands): elevation below 2,500 feet; January temperatures average 54°F; July temperatures average 69-74°F; coastal fog is a common occurrence in the summer and at times in the winter; rainfall averages between 10-35 inches per year; 85-90 percent of the rain falls during the six winter months October through March; snow is rare; chaparral is the most widewpread form of vegetation.

(2) DENSITY

The following parameters have been grouped together in order to define density:

Total Number of Systems
Acres per System
Population Distribution (Age)
Number of People in System

The assumptions regarding the above parameters have been made yielding six density classifications.

Density Classification	High (H)		Medium (M)		Low (L)	
Parameter	A	B	A	B	A	B
Total number of systems	<20	>20	<20	>20	<20	>20
Acres per system	<1	<1	1-5	1-5	>5	>5
Population distribution (Age)			NORMAL			
Number of persons per system			2.5			

RISK EVALUATION TECHNIQUES: ROBERT COOPER

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: Morrison

Dr. Cooper, I am curious. You, of course, ran this study in a particular region in the country with people who were familiar with the local operations and geography. Do you believe the same sort of results would occur if you had gone to the Gulf Coast area or the New England area or the Wisconsin area?

A: Cooper

We considered that and mentioned it in the paper, as somewhat of a disclaimer, that this data would not have a universal application. Obviously, there will be some regional differences. These results might well be applicable in the United States, but certainly in third world countries you would not want to apply these risk values. The more information that becomes available, such as in meetings like this, ought to improve these estimates. My feeling is that these results are probably equitable throughout the U.S. Maybe some parasitologist would think differently of the Gulf States.

Q: Pipes

This is really an answer to Buck's question. There was the same type of thing done on the Chicago south end of Lake Michigan wastewater disposal study by the Corps of Engineers project there. And so it's been done in places besides California.

Q: Audience (Sue Lewis)

I am excited to be here to hear these papers. I was involved in a 208 project contracted to the State Health Department. I initiated one of these matrix exercises. In principle it is very similar to what you are talking about, but instead of choosing only the biological aspects/factors, we considered factors from five groups, including economics, biology, technical facilities part and politics. From each group we broke down several of the parameters and did the exercises. We applied those to the kinds of alternatives we considered feasible. It turned out quite promising and has already been applied in the 208 project. Thank you.

A: Cooper

As I understand it she is describing the use of a similar method in a 208 project in a number of Oklahoma cities. A major problem with decision-making is dealing with a large number of variables (problems) with one set of experts. Some difficulties certainly can occur. It is said that the human mind can only handle a few variables at a time, yet frequently you start with 20 or 30. The problem level has to be limited otherwise you just can't deal with it. In the case of this method you first ask the experts how many pieces of information they would need to make a decision, limiting such to about 12 items. They are also asked to prioritize the list. Then one must discern the impact of deleting the lower priority items. For example, if you removed the lowest two could one still make an acceptable problem occurrence estimate? In this way, the method is manageable.

Q: Rycheck

Dr. Cooper, it appears to me you are expanding, going statewide and maybe nationwide. My question is how do you plan to determine whether your results actually reflect truth? How do you know if these results really have any meaning?

A: Cooper

Well, I hate to say "the proof is in the pudding." I suppose, basically, we are doing the same thing now anyway (i.e., making judgments) only we are doing it in a less organized way. I really can't answer your questions directly, but time and experience will indicate the correctness of our estimates. The overall opinion of the experts involved in this exercise is that the use of these low water requirement toilets does not pose a public health problem. Perhaps for an individual family using them, there may be a household case of diarrhea but that wasn't considered by the experts as a major public health problem.

A significant factor that might affect the public health potential of these systems is crowding. You cannot use these systems in dense population areas -- just because of odor if nothing else. As stated previously, the "proof of the pudding" will be practice in use.

These waste disposal systems are presently in limited use in California. We've talked to people who are knowledgeable about these operations and most feel that the public health risk is relatively low. Since performing this risk estimate exercise, I certainly am comfortable about them. It remains, however, an evaluation and best estimate based upon expert opinion which, I am sure, makes a bench scientist rather nervous.

Q: Little

I don't think I quite understood how the needs of value were arrived at, whether the experts who were asked for this were given a special set of circumstances under which these values to any particular problem or is this in general or...?

A: Cooper

There were not real scenarios developed around each one of these conditions. What we did in getting these mean values, R, (the probability of a public health problem occurring) was to assume that the small r values (see equation 2) acted independently. This assumption is not really correct; however, we tried to group conditions that were as independent as possible. In this way, the experts could make a problem-occurring judgment just based, for example, on climate independent of land slope, crowding, etc., for the various disposal systems. Am I answering your question or not?

Q: Little

Well, I am thinking about particular health problems, infections with particular organisms gives a mean severity value for those parasites. Anyway, I think they have to define a certain set of circumstances because unlike typhoid or salmonella where once an infection is established, the severity may not necessarily depend on infective dose with ascaris or hookworm and animal parasites. The severity of infection is related to the infective dose since they don't multiply generally in the body. So it is related to other sets of circumstances that vary in different regions depending on the soil type, if they are soil transmitted parasites. For example, in one area in Colombia that I examined, 50 percent of the people in this area were affected with hookworm but there was not one case of hookworm disease.

A: Cooper

This was the sort of thing that was pointed out very strongly by the parasitologist who was involved in this exercise. He pointed out the lack in universality in applying these numbers, certainly in countries where the morbidity of disease would be very high. I am certain that in making judgments concerning the probability of hookworm disease occurring, the expert in parasitology considered the relationships between infectious dose and the occurrence of clinical disease. The job of the non-medical experts was to make estimates of the probability of a problem occurring, not the severity of the problem. Physicians developed the mean disease severity values. In developing these values, they were to consider the severity of a "full blown" case of disease in such terms as lethality, treatability, discomfort, etc., but not in terms of disease morbidity. Thus, in the case of hookworm the severity would be based upon an actual clinical case and not upon the probability of a case occurring.

ENGINEERING CONTROL MEASURES

CONTROL MEASURES - SUBSURFACE DISPOSAL

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ABSTRACT

This paper reviews the current state of art of design of subsurface disposal systems with emphasis on design to avoid microbial contamination of surface or subsurface receiving waters. Likely causes of hydraulic or treatment failures in subsurface systems were delineated. Alternative designs to mitigate against these failures were outlined. Technical questions that need answers before effective designs can be provided to insure against potential microbial contamination were described.

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CONTROL MEASURES - SUBSURFACE DISPOSAL

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INTRODUCTION

The septic tank-soil absorption system (ST-SAF) has been implicated by a number of investigators as an important source of microbial contamination of ground and surface waters (1, 2, 3, 4). Normally, this contamination occurs through the transmission of untreated wastewater through the soil to a ground or surface water source. It may also occur through the surfacing or direct discharge of untreated effluent to a receiving water. Kreissl (5) has described the likely sources of microbial pathogens in domestic wastewater and has traced their fate in conventional ST-SAF systems. To be acceptable, the system must perform two important functions. First, it must allow complete infiltration of wastewater into the seepage system at all times. Second, it must provide effective treatment of the wastewater so that receiving ground or surface waters are not contaminated at any time. Failures of the system, therefore, can be attributed to either hydraulic failure or incomplete treatment, or both. Table 1 outlines the major causes of ST-SAF failure, classified in accordance with hydraulic or treatment problems.

SYSTEM FAILURES

Hydraulic Failures

Movement of wastewaters into and through the soil is essential for achieving successful subsurface disposal. The flow of wastewater from the distribution system must infiltrate the interface between the soil and the distribution field, percolate through the underlying soil, and be transmitted away from the absorption field. Soil physical theory can be used to describe and predict rates of liquid movement but is currently limited in practical usefulness because (a) the theory of fluid flow in soils is complex requiring substantial mathematical analysis and (b) the theory has been derived primarily for isotropic homogeneous porus media which does not resemble natural soils. Excellent attempts to simplify flow theory may be found, however, in the literature (6, 7, 8).

Failure of soil absorption fields to infiltrate and percolate water away often lie in a proper understanding and assessment of the infiltrative resistance of the soil-crust interface which develops after a period of system operation. Percolation tests, which are normally used to assess soil permeability are, physically, poorly defined and can not be used to estimate unsaturated flow which normally occurs in properly functioning systems. The development of hydraulic conductivity characteristics for soils and the measurements of equilibrium crust resistances which develop in mature soil absorption fields will greatly facilitate proper assessment and design of infiltrative surfaces. Details of this design approach are described elsewhere (7, 9, 10).

TABLE 1

MAJOR CAUSES OF SEPTIC TANK-SOIL ABSORPTION FAILURE

	Hydraulic Failure	Treatment Failure
Site Conditions	.Low Permeability .Poor transmission from site .Poor surface drainage .Layering of soil .Steep slopes	.Very high permeability .High ground water table .Shallow, creviced bedrock
Design	.Overestimate of infiltrative capacity of soil .Underestimate of Flow .Insufficient site information .Poor distribution design .Poor pretreatment design	.Inappropriate distribution .Misapplication of site information
Construction	.Smearing or compaction of soil .Poor site preparation .Poor distribution layout	.Poor distribution layout .Poor site preparation
Maintenance	.Inappropriate septic tank pumping .Poor plumbing maintenance (excess water) .Poor maintenance of pretreatment devices	.Poor maintenance of pretreatment devices
Enforcement	.Use of illegal systems .Use of failed systems	.Use of illegal systems .Use of failed systems

In addition to the natural physical and biological clogging that occurs in mature soil absorption fields, severe clogging may be accelerated by the overflow of solids and grease from improperly maintained septic tanks, grease traps, or other pretreatment processes which precede the soil absorption field. This clogging is unpredictable in design and must be eliminated through proper maintenance. Soil compaction or puddling is another major cause of soil absorption field ponding (11, 12, 13, 14, 15). Both compaction and puddling of wet clayey soils are the result of poor construction practices.

Once water is conveyed through the infiltrative surface, it must be effectively carried away from the disposal site. The poor transmission of water away from the disposal field may result in the development of a perched water table which eventually may penetrate the infiltrative surface of the soil absorption field. Although, not common in single family ST-SAF systems, Otis has reported the occurrence of this problem in a large community soil absorption field (16).

Hydraulic failures have also occurred in areas where internal soil drainage is poor. Included within this group of almost unlimited drainage problems are free water tables, water tables over artesian aquifers, perched water tables, and lateral moving groundwater. Sites on steep slopes may produce hydraulic failures if layering of the soil or other geological formations intercept the surface.

Finally, hydraulic failures will result from hydraulic overloads to the ST-SAF. Hydraulic overloads may be due to poor estimates in design flow rates, poor plumbing maintenance, or poor siting of the system in areas receiving large quantities of surface drainage.

Treatment Failures

The soil serves not only as a means to transmit the wastewater away from the site but also provides treatment of the wastewater. Kreissl (5) has described the effectiveness of septic tanks, the absorption field, and the soil in removing pathogens contained within domestic wastewater.

The clogged layer and the aerated zone immediately below it at the infiltrative surface are very effective in removing pathogenic organisms from wastewaters (17, 18). This zone not only harbors an abundant population of microbes which compete with pathogens for survival but also acts as a flow barrier, producing unsaturated flow in the soil below. Experimental studies have shown that the flow regime is critical to effective pathogen removal. Long detention times within the soil and effective contact between the soil and water will result in excellent purification (17, 18, 19, 20). Unsaturated flow will produce these desirable conditions; especially, in the structured soils containing large pores such as root and worm channels and planar voids because the wastewater is forced into the finer pores.

Site conditions which may result in unsatisfactory removal of microbial pathogens include the very permeable soils, which provide very short detention times and poor soil-water contact, and the shallow soils in which there is insufficient detention time for effective pathogen destruction. Creviced bedrock overlain by shallow, very permeable soils, act as channels to carry untreated wastewater with short soil-water detention times to deeper ground water sources. A surface drainage condition which results in the intrusion of water into the drain field area producing a saturated condition below the absorption field or overloading the soil absorption field will also result in poor microbial destruction.

In summary, any situation which results in short travel times and little contact between the soil and the wastewater will result in unsatisfactory destruction of microbial pathogens. This may be brought about by poor siting, poor design, poor construction, and insufficient maintenance of pretreatment facilities.

DESIGN TO MITIGATE AGAINST SYSTEM FAILURE

The key to successful design of the subsurface soil disposal field lies in the appropriate application of a system to fit the site. Currently, soil surveys classify sites and soils as "slight", "moderate", "severe", and "very severe" which greatly limit the flexibility in designing a system to fit the site. An alternative procedure is to define general criteria for adequate soil disposal and treatment to be followed by the description of the system designs which would be suitable for a particular set of soil and site conditions. In this concept, every soil and site would be considered a problem soil or site (10). It is on this basis that the following section is written.

The emphasis will be to key the system failure to the site or soil condition and to recommend a system design to mitigate against that failure. To this end, Figure 1 has been constructed, after a conceptual soil-site and design analysis developed by the Small Scale Waste Management Project (17). Note that "Acceptable" conditions represent those site-soil conditions which will accommodate conventional soil absorption system design. Although acceptable conditions will be dependent to some extent upon the specific site conditions, they are normally limited to permeable soils with relatively deep groundwater and bedrock levels on limited slopes.

Septic Tank or Pretreatment

The primary purpose of the septic tank is to keep both settleable and floatable solids out of the soil absorption field. To this end, the tank must be designed to prevent short circuiting and solids re-entrainment. The details of design and performance of septic tanks are found elsewhere (11, 21, 22, 23). Although individual septic tanks are normally overdesigned and present few problems with respect to performance, community septic tank design is not well documented. Of greatest concern in septic tank performance is the maintenance of the unit. If sludge pumping is not performed when required, solids and greases may overflow resulting in absorption field clogging which will lead to hydraulic failure of the system.

Grease traps which precede soil absorption fields are designed to prevent absorption field clogging with heavy grease loads. Design and performance of these units are described elsewhere (11, 23). Maintenance of grease traps is paramount to good performance of the unit and successful operation of the soil absorption field.

Soil Permeability

The successful disposal of wastewater into the soil is dependent upon the permeability of the soil. There is some minimum infiltration rate which is required to allow economic absorption of wastewater within a reasonable area. On the other hand, very permeable soils may lead to inadequate wastewater treatment. Currently, "acceptable" permeabilities are established by State regulatory codes and are based on experience with on-site disposal systems. As indicated earlier, the use of percolation tests alone are normally unsatisfactory for establishing the

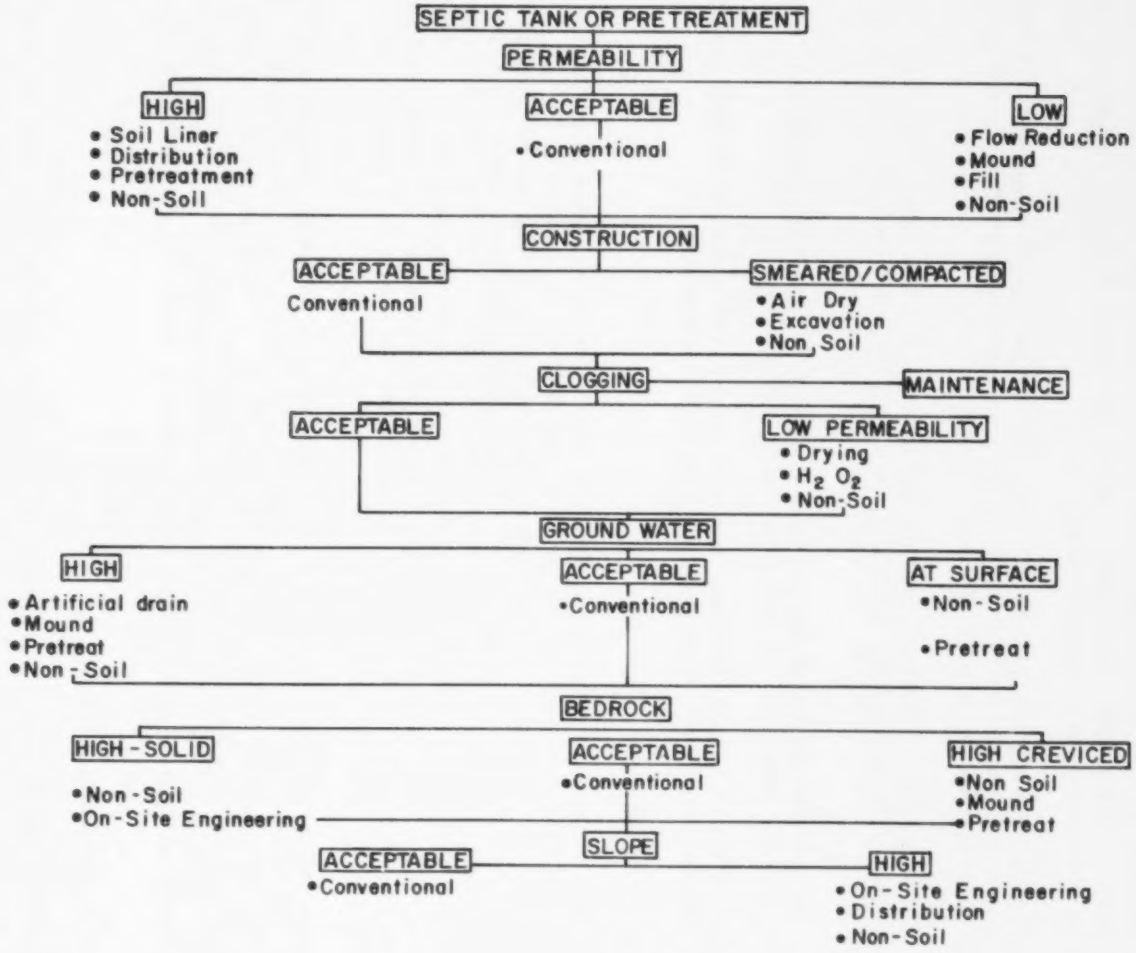
SEPTIC TANK OR PRETREATMENT
PERMEABILITY
HIGH
• Soil Liner
• Distribution
• Pretreatment
• Non-Soil
ACCEPTABLE
• Conventional
LOW
• Flow Reduction
• Mound
• Fill
• Non-Soil
CONSTRUCTION
ACCEPTABLE
Conventional
SMEARED/COMPACTED
• Air Dry
• Excavation
• Non, Soil
CLOGGING
MAINTENANCE
ACCEPTABLE
LOW PERMEABILITY
• Drying
• $H_2 O_2$
• Non-Soil
GROUND WATER
HIGH
• Artificial drain
• Mound
• Pretreat
• Non - Soil
ACCEPTABLE
• Conventional
AT SURFACE
• Non-Soil
• Pretreat
BEDROCK
HIGH - SOLID
• Non-Soil
• On-Site Engineering
ACCEPTABLE
• Conventional
HIGH CREVICED
• Non Soil
• Mound
• Pretreat
SLOPE
ACCEPTABLE
• Conventional
HIGH
• On-Site Engineering
• Distribution
• Non-Soil

Figure.1.

level of "acceptability" of a soil to handle a given wasetwater discharge. More rational approaches are described in a number of publications (7, 8, 9, 10).

Regardless of the soil permeability, several design features should be considered for all systems.

. Landscaping of the system should be provided so as to divert all surface waters away from the soil absorption field. No storm water or foundation drains should be directed to the septic tank.

. Evapotranspiration within the soil absorption field should be optimized; thereby, reducing the amount of wastewater that must be absorbed by the soil. This can be accomplished best with shallow trenches.

. Distribution systems should be designed to fit the characteristics of the soil and site. Proper design will mitigate against both hydraulic and treatment failures.

. Construction of the disposal field should be controlled carefully to avoid soil compaction, smearing, or inappropriate layout.

. Maintenance of pretreatment systems and plumbing should be enforced to ensure against absorption field clogging and hydraulic overloading.

Soils with "Acceptable" Permeability

The design of soil absorption systems for soils of acceptable permeability is relatively straightforward and is well documented (7, 11, 17, 23, 24, 25). Shallow trenches or beds are most commonly used and are sized on the basis of soil texture and percolation rate. Trenches are more desirable than beds since they are less susceptible to damage during excavation (17, 25, 26). Effluent distribution may be provided by gravity flow, dosing, or uniform pressure distribution. Gravity distribution is most commonly used in conventional systems, although uniform pressure distribution or dosing may be desirable in the more permeable soils (see Soils with High Permeability).

Proper site evaluation, design, and construction of these conventional disposal systems should result in successful removal and destruction of pathogens. Start up of soil absorption fields in sandy soils may be of some concern, however. Until a clogging mat develops, short circuiting of effluent may be a potential cause of pathogen contamination. Control of this problem, which may occur over a relatively short period of time, is described in more detail under "Soils with High Permeability".

Soils With Low Permeability

As soil permeability decreases, required soil absorption trench or bed infiltrative area increases to a point where a further increase in area is not feasible. At that point, several alternatives are available;

- Flow reduction,
- Mounds,
- Fills,
- Non-soil alternatives.

From a microbial contamination point of view, the system must be designed to prevent hydraulic failure. Hydraulic failure may be eliminated by decreasing flow rates of the wastewater to a point where the soil will accept it all of the time. Flow reductions may be achieved through in-house water conservation (11, 17, 27). The toilet, bath, and laundry events in the home account for over 75 percent of the total wastewater flow. The use of water conserving plumbing and low flush toilets may reduce flows by as much as 30 percent (27). Unfortunately, it is not possible to rely on water conservation methods unless the passive type (no involvement by homeowner) are employed and proper management and control prevails.

A second alternative to low permeability soils is the engineered mound system (11, 17, 28). In slowly permeable soils, the mound serves to improve absorption of the effluent by utilizing the more permeable topsoil and eliminating construction in the wetter and more impermeable subsoil, where smearing and compaction are often unavoidable. The mound consists of a suitable fill material, an absorption area, a distribution network, a cap, and top soil. Critical to the successful performance of the mound are the selection of proper fill material and design of the distribution network. Fill materials currently used or under investigation include medium sand, sandy loam, sandy-sandy loam mixtures, and bottom ash. Distribution networks include both pressure and gravity systems.

Since the fill material is critical to both hydraulic and treatment capacity of the mound, efforts should be made to conduct particle size analysis prior to placement. Recommended fill material for the Wisconsin mound is a medium sand which includes 25 percent or more of coarse, very coarse, or medium sand (>.25 mm), less than 50 percent fine or very fine sands (<0.25 mm) and not more than 10-15 percent fine or very fine sands (<0.05 mm) (11, 17, 28). A recent study of 33 mound systems in Wisconsin indicated that the failure of two mounds was due to poor fill material (29). One contained a loamy sand and the other a sandy loam with an average uniformity coefficient of 50.

In Pennsylvania, 5 to 15 percent clay (by weight) in the sandy fill material is required based on the assumption that clay will increase purification of wastewater and reduce the depth of soil needed for renovation below the mound (30). Studies of eight field mounds in Pennsylvania demonstrated extensive failures due to ponding and uneven distribution of effluent (30). Although the high clay content was not directly cited for these failures, it was evident that sorting of the fill material resulted in pockets of stratified fine materials.

Distribution of wastewater must be uniform to ensure unsaturated flow within the fill system. The Pennsylvania studies concluded that the chief cause of failure of the mounds was due to the uneven distribution of wastewater produced by the gravity flow network that was employed (30). This report recommended, therefore, that, in order to prevent hydraulic failure of the mound, pressurized distribution systems should be used to allow for uniform distribution of the wastewater to the fill. The Wisconsin mound provides pressurized distribution and this system, coupled with a proper medium sand fill, has been successful in overcoming hydraulic failures. It should be noted, however, that hydraulic loading to the fill must be commensurate with the design infiltration rate of the fill. The Wisconsin study (29) indicated that many of the systems tested produced dosing rates (expressed as volume/area of absorption bed/dose) which exceeded acceptable limits; thereby, likely producing a saturated flow condition within the fill. Dosing rates can be adjusted easily, however, by proper setting of pumping volumes and times.

In addition to fill and distribution network, the mound absorption bed or trench must be designed to minimize wastewater seepage from the base of the mound. For soils with low permeabilities, beds should be narrow and extended along the contour as far as possible. Fill depth from absorption trench to natural soil is normally 12 inches. The basal area of the mound must be sized so that it absorbs the wastewater before it reaches the mound perimeter. The infiltrative rates which are recommended for the slowly permeable natural soils to size the basal area are conservative and assume that a clogging mat forms at the fill/natural soil interface (11, 17, 28).

A third alternative in low permeability soils may be the fill system. The use of fills is normally restricted to sites where unsuitable surface soils may be stripped away without damaging the more permeable underlying soils (11, 25). Fills are limited to sites where the underlying soils are sands or sandy loams and the seasonly high water table or bedrock surface is not within one foot of the sand or sandy loam surface. If three to five feet of sand or sandy loam is available between the water table or bedrock, a fill system would not be required.

Once the impermeable layer is stripped away, two to four feet of a sandy material similar to the underlying sand or loamy sand is added. A trench or bed system is constructed within this fill. Design of fill systems are identical to conventional trenches or beds except that a minimum separation distance from absorption bed or trench to the edge of the fill area of five feet should be maintained to allow for sidewall absorption and lateral movement of wastewater. Uniform distribution by a pressurized network is strongly recommended to avoid localized saturated flow.

Soils with High Permeability

Conventional trenches or beds should not be used for rapidly permeable soils (faster than 1 min/in) since wastewater retention times may be very short and soil-wastewater contact may be poor. Disposal to these highly permeable soils may be possible, however, employing several alternative designs including

- Soil liners,
- Uniform distribution,
- Wastewater pretreatment.

One method used to overcome problems of highly permeable soils is the soil liner. The native soil is replaced by a suitably thick (greater than two feet) layer of loamy sand or sand textured soil (11, 25). With this liner in place, the design of the system can follow the conventional trench or bed design using application rates consistent with the soil liner.

The rapid infiltration of wastewater into highly permeable soils may be reduced significantly by the application of many small doses of wastewater to the absorption field over the day. This will result in an unsaturated flow condition and should produce more favorable conditions for microbial destruction and removal within the soil matrix (11, 17, 26, 31). Even more desirable, however, would be the uniform application of wastewater over the entire bottom area of the system to minimize local overloading and the depth of ponding after each dose. In this manner, the soil is more likely to remain unsaturated, even during initial start-up periods when no clogging mat is present. Uniform application, normally by pressurized networks, is highly recommended for soils with very rapid (1/min/inch) as well as rapid (1-10 min/in) permeability (11, 17,31). Additional field research is needed to provide supportive evidence that uniform distribution in the very permeable soils will serve to effectively remove pathogenic organisms to a safe level.

The pretreatment of wastewaters, prior to subsurface discharge to very permeable soils, may represent another practical alternative for on site treatment. Currently, pretreatment methods over and above septic tanks or grease traps have not been employed for this type of application.

Theoretically, the mound or any type of sand filter, with proper sand size and loading rate, should be able to treat the waste satisfactorily prior to discharge to the permeable soil below. Loading of wastewater to the filter or mound and its proper distribution are critical, however, if satisfactory pathogen reductions are to be achieved. For example, Green & Cliver (17, 20) reported complete removal of 5.5 logs of polio virus type 1 in 24 inches of sand loaded at 1.25 gal/day/ft^2(5cm/d) based on laboratory column studies with a Plainfield loamy sand (0.2mm). At a tenfold increase in loading rate, some viral breakthrough was observed. It was also noted that the sand was less retentive of virus at lower temperatures (6-8°C). Ziebell, et al (17, 19) found similar results for bacterial indicators in sand columns. These results would suggest that 24 to 36 inches of medium sand should remove microbial pathogens effectively if loaded at rates equal to or less than 1.25 gal/day/ft^2 (5 cm/d).

Recent field data for mounds (29) indicate that bacterial indicators were present at the basal area (below fill) for most of the systems studied. Total coliform and fecal coliform attenuation in the fill was high, (greater than 99 percent) but fecal streptococci and Pseudomonas aeruginosa removals were poor. This breakthrough was attributed primarily to a "quasi-saturated" flow through the mounds due to high dosing rates (expressed as gal/dose-ft^2 of trench or bed area in the fill). Many of the mounds that were surveyed produced high dosing rates as compared to rates recommended for the medium sands that were employed. (It should be noted that "dosing rate" is an instantaneous application rate as compared to the average application rate which is calculated over the entire day.) No clogging mat development was apparent at the fill-trench or bed interface for the mounds studied. Recent, yet unpublished, field data on the fate of the virus below mounds (Cliver 32) suggests that occasional breakthrough of virus may occur also with the current mound design.

Intermittent sand filters will provide indicator organism attenuation of 2-4 logs at the high rates of application (5 gal/day/ft^2, 20 cm/d) normally used (11,17). Lower loading rates and finer sand sizes will produce more effective attenuation of indicators. The laboratory studies by Green & Cliver (17, 20) and Ziebell et al (17, 19) would suggest that loadings on medium sand below 1.25 gal/day/ft^2 should remove most pathogens effectively. Additional field research on the fate of selected bacteria and viruses in mounds and sand filters is required before confident design can proceed, however.

Other pretreatment systems also might be considered for this application. Disinfection processes have been successfully employed on-site for sand filtered effluents (11, 17). Among the devices currently in use are stack feed calcium hypochlorite feeders, iodine saturators, and small UV irradiation systems (11 , 17). Control of calcium hypochlorite feed has been of some concern, as has been the resultant residual chloramine. UV irradiation requires careful maintenance and results in no residual. Iodine saturators are more complex than stack feed devices to use, but are effective.

Finally, some consideration should be given to wastewater segregation in the home. Separation of the toilet wastes from the remaining household wastewaters will effectively reduce potential pathogen levels in the remaining grey water fraction. The black water must be handled separately and does represent a high risk pathogen source. Grey waters do contain substantial numbers of indicator bacteria (11, 17, 27) but the health hazard of this waste is presently unknown.

Soil Clogging

The clogging of the infiltrative surface of a soil absorption field may be caused by poor construction practices or due to physical/chemical/biological factors associated with the application of wastewater to the soil absorption field (7, 17, 24, 26, 33). This latter cause of clogging is a complex phenomona and depends to a large extent upon the characteristics of the soil and the applied wastewater, the rate of wastewater application, the method of application, and the soil temperature. As discussed earlier, a certain degree of clogging at the infiltrative surface is desirable since it produces an unsaturated flow condition in the underlying soil. Yet, excessive clogging (or the development of a clogging resistance substantially greater than predicted) may result in hydraulic failure of the system.

Designing to accommodate for or to encourage the development of a clogging mat is not well documented and is currently based on designer experience with the soil texture, structure, depth, and other site features. The effects of construction techniques on the development of clogged infiltrative surfaces is better documented (11, 12, 13, 14, 15).

Construction Practice

Poor construction techniques may result in soil compaction, puddling, or smearing, normally leading to early failures of soil absorption fields. Over the years, a number of guidelines have been developed to mitigate against this type of failure.

- Care must be taken to keep construction equipment off the absorption area until construction begins.
- Trenches, rather than beds, are preferable in soils with significant clay content (greater than 25 percent by weight) because the equipment can straddle the trenches; thereby, avoiding smearing and compaction of the soil.
- Trench spacing should be wide enough to allow operation of construction machinery.
- Trench widths should be large enough to accommodate excavation buckets to move around without excessive sidewall compaction.
- When excavating in clay soils, the moisture content should be below the plastic limit of the soil so as to avoid compacting or smearing.
- Excavation equipment which produces a scraping action should be avoided.
- All infiltrative surfaces should be left with a rough, open surface and any smeared or compacted surfaces should be removed. Work should be scheduled only when the infiltrative surface can be covered in one day to avoid windblown silt or raindrop impact which can clog the soil.
- Backfilling of gravel or crushed rock onto the infiltrative surface should be accomplished carefully. The fill should be laid in by backhoe or

front end loader.

Rehabilitation

The rehabilitation of clogged soil absorption fields is dependent upon the cause of the failure and its incidence. A detailed description of rehabilitation strategies is described elsewhere (11). In general, clogging failures are most often due to system overloads, insufficient maintenance, or poor construction procedures. Once the cause is delineated, alternatives for rehabilitation may be reviewed and appropriate action taken.

Where damage to the soil has taken place due to poor construction techniques, reconstruction of the site or its relocation may be necessary. Severe physical/chemical/biological clogging due to wastewater quantitative or qualitative overload may be alleviated by providing a resting period in order to rejuvenate the clogging mat (17, 33).

Resting may be accomplished by the construction of a new field which will continue to be used in conjunction with the existing field to provide periods of rest and dosing (usually 6 to 12 month periods). Alternatively, the septic tank could serve as a holding tank during the rest period, but this would require frequent, costly pumping.

The application of oxidizing agents, particularly hydrogen peroxide, are being used in some soils to oxidize the clogging mat and, thereby, restore the infiltration capacity of the absorption field (17, 34).

Groundwater

Saturated soil is normally considered to be unsuitable as a medium for waste disposal and treatment. Inadequate removal of fecal indicators and virus in saturated soils has been reported (35, 36, 37). Furthermore, the flow of these often static aquifers located near the gound surface is often unpredictable.

Included in this definition of saturated soil are both ground water and perched water tables. The often seasonal perched water table is dynamic, moving primarily in a downward direction through a slowly permeable horizon. This flow offers substantially greater opportunity for contact between soil and effluent and, thereby more effective removal of microbes than the more static groundwater system (17, 19, 20). Nevertheless, the use of groundwater aquifers close to the ground surface as a principal medium for waste disposal includes too many health risks to be generally acceptable.

The determination of acceptable treatment of wastewaters prior to discharge into groundwater is described in more detail by others at this conference. Suffice it to say that such a determination is no simple task. Among the complicating factors are predicting the flow regime, estimating the dispersion of the effluent into the groundwater, and determining the assimilative capacity of the groundwater system for microbial contaminents.

The basic design consideration in soils where high water tables exist, therefore, is to provide appropriate treatment consistent with water quality objectives. In order to insure acceptable removal of microbial pathogens, the wastewater must be either pretreated or else sufficient soil must be provided between the infiltrative surface and the high water table elevation. The former may be accomplished through the use of a variety of treatment processes including sand filtration and disinfection. Mounds, constructed above ground level, also may be considered as pretreatment devices in this application. Finally, when conditions are appropriate, the area may be drained artificially so that conventional trench or bed systems may be used.

Wastewater Pretreatment

The treatment of wastewater prior to discharge to soil with a high water table must achieve target levels of microbes consistent with groundwater (or perched water) quality objectives. Potential treatment processes were described under the section entitled Highly Permeable Soils. Disinfection alternatives will produce effluents that will likely meet most water quality objectives, but proper management of small systems is a potential drawback. Waste segregation practice with separate black water handling is also a potentially promising alternative. High fecal indicator counts in grey water (27) do raise some questions, however, regarding potential health hazards of this waste fraction. Further evaluation of grey water is needed to evaluate this alternative better.

Mounds for High Water Tables

The use of mounds as treatment devices for wastewater has been described earlier (Highly Permeable Soils). Current mound designs for soils with high water tables recommend 1 foot of fill plus 2 feet of permeable soil between the infiltrative bed and the high water table elevation. There is evidence (17, 18, 19, 20) that this depth of fill and soil should remove bacteria and virus effectively in wastewaters loaded at rates equal to or less than 1.25 $gal/day/ft^2$. The problems associated with high dosing rates, even with uniform pressure distribution, were described in an earlier section of this paper. As was the case for mounds designed over slowly permeable soils, the bed or trench should be made narrow and extend along the contour as far as possible. This will prevent problems with the development of a groundwater mound under the fill with resultant interception of the fill with subsurface water.

Artificially Drained Systems

High water tables that limit the use of trenches or beds can sometimes be lowered artificially to permit the use of these disposal methods. Vertical drains, curtain drains, and underdrains are commonly used subsurface drainage techniques. Soil and site conditions dictate the method which is selected (11).

Successful design of artificially drained systems depends upon the correct diagnosis of the drainage problem. The source of the groundwater and its flow characteristics must be determined to select the proper method of drainage. Particular attention must be given to soil stratification and groundwater gradients. There are an unlimited variety of subsurface drainage problems, but the most common ones can be grouped into four general types which include free water tables, water tables over artesian aquifers, perched water tables, and lateral groundwater flow problems.

Soils with high water tables that may be practical to drain to make a site suitable for a trench or bed system are ones having (1) shallow perched water tables, (2) lateral groundwater flow, or (3) free water tables in coarse textured soils. Soils that are saturated for prolonged periods are not practical to drain.

Alternative off site methods would be recommended in these cases.

Health guidelines establishing minimum distances between the disposal area and intercepting drain lines have not been well documented. There is evidence, however, that microbes can be transported long distances in a saturated flow condition. (36, 37). This, coupled with the potential existence of groundwater mounds under the soil absorption fields, create concerns over the lateral transport of microbes to these drainage systems. Since the use of underdrains for on site disposal has been very limited, other alternative methods not requiring drains should first be considered.

The procedures for the site evaluation required for proper drain selection and the design and construction details for these systems are presented elsewhere (11). A tabulation of alternative drainage methods for various site characteristics is presented in Table 2.

TABLE 2

DRAINAGE METHODS FOR VARIOUS SITE CHARACTERISTICS (11)

Site Characteristics	Drainage Problem	Drainage Method
Saturated or mottled soils above a restrictive layer with water source located at a higher elevation; site usually sloping	Lateral flow	Curtain drain Vertical drain*
Saturated or mottled soils above a restrictive layer; soil below restricted layer is unsaturated; site is level or only gently sloping	Perched water table	Underdrain# Vertical drain*
Deep uniform soils mottled or saturated	Free water table	Underdrain#
Saturated soils above and below restrictive layer with hydraulic gradients increasing with depth	Artesisn-fed water table	Avoid

*Use only where restrictive layer is thin and underlying soil is reasonably permeable.

#Soils with more than 70% clay are difficult to drain and should be avoided.

Bedrock

A distinction must be made between creviced, permeable bedrock such as limestone or shale. The design objective for disposal systems is substantially different for these two situations.

When the soil depth to permeable bedrock is shallow, inadequate treatment of wastewater will occur in the thin layer of soil on top of the bedrock. The obvious solution is either pretreatment or the addition of sand fill to the existing soil so that the vertical distance of unsaturated flow to the permeable bedrock is adequate for microbial removal (e.g., a mound).

Pretreatment methods have been discussed in a previous section and would be equally applicable for this situation. The use of engineered mounds in shallow soils over permeable bedrock is described elsewhere (11, 28). The mound is designed primarily as a treatment device and, thereby, must be sized so as to provide sufficient vertical unsaturated flow to the bedrock. If the top soil is permeable, there should be no flow problems below the mound and either trenches or beds (normally square) can be employed. Again, dosing rates are important to insure unsaturated flow through the fill.

The presence of shallow, impermeable bedrock presents a different type of disposal problem. The prediction of lateral flow patterns in generally hilly terrain is very difficult. No general guidelines can be provided since local soil conditions, depth of bedrock, and topography will dictate design. Generally, off site methods are to be recommended. Where site conditions are favorable, some type of mound system may be applicable to provide both treatment and distribution for the wastewater.

Slopes

Limiting slope is normally considered a function of the soil permeability. Of major concern is the hydraulic failure of systems on slopes where effluents may surface downslope from the system. This is a function of the soil profile and the wastewater application rate to the absorption field. If the soil profile does not contain slowly permeable layers which would normally create lateral flow along the impermeable horizon, effluent should move in a predominantly vertical direction. Under those favorable conditions, there is no reason why systems can not be constructed on slopes which would be limited only by access for excavation equipment. (11).

Since wastewater distribution to absorption fields on slopes is critical, considerable care must be given to design of the distribution system. In general, trenches, rather than beds, are recommended for slopes in excess of 5 percent. The wastewater should be distributed equally along the trench infiltrative surface. This could be accomplished by pressure networks, but such systems would be very difficult to design. Of more practical application, are serial distribution systems. Further research on wastewater distribution design for sloping sites is needed, however, to corroborate current design practice.

MANAGEMENT

Successful control of public health hazards that might result from the subsurface disposal of wastewater is dependent upon proper management. An effective management program must provide both technical assistance and strong enforcement. The management entity must include validation of site evaluation, review of system design, supervision of system construction, certification of operation and maintenance, assistance in rehabilitation, and monitoring and enforcement. Many system failures must be ultimately attributed to the management function, although technical information on siting, design, and construction are still needed in many vital areas.

The types of management entities for on-site systems and small communities and guidelines for planning and development of these programs is described in detail in several publications (11, 17, 38).

CONCLUSIONS

Subsurface soil absorption systems, when properly sited, designed, constructed, and maintained under a strong management function, can provide many years of effective and acceptable wastewater disposal for individual homes or small communities. System failure may be attributed to either hydraulic or treatment failures due to poor siting, poor design, poor construction, or poor maintenance. The key to successful performance is proper site evaluation. Every site should be considered a problem site and system designs should be classified as suitable for particular groups of soil and site conditions.

Technical solutions are available for a large number of adverse soil and site conditions. Yet, there are still many unanswered technical questions remaining. Included within this group of unanswered questions are the following:

- How can we improve our predictions of acceptable hydraulic loading to selected soil and site situations?
- Uniform distribution or dosing is very effective in achieving acceptable microbial removals in highly permeable soils; how important are these methods of wastewater distribution in finer textured or unstructured soils?
- What method of wastewater distribution are most effective at sites with excessive slopes?
- What type of hydraulic regime exists under large soil absorption beds for different soils? Can one develop better predictive models for this flow system so that large absorption field design may be improved?
- Can better predictive models be developed for flow regimes that exist for soil absorption fields located in soils which must be drained artificially?
- Can we better model the behavior of virus in subsurface soil absorption fields or their technical alternatives?
- What are the health hazards associated with grey water?

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CONTROL MEASURES - SUB-SURFACE DISPOSAL: WILLIAM BOYLE

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: Morrison

Dr. Boyle, from your own experience and the literature, can you comment on the evapotranspiration aspect of these systems? Are there ways of optimizing them?

A: Boyle

Yes. Normally, the best way to optimize evapotranspiration in a conventional absorption field is to employ shallow beds or trenches. In cold climates, one should have at least six to twelve inches of cover over the system. The shallow system, therefore, will take advantage of any transpiration and evaporation upward. In past years, treated building paper was used to prevent backfilled soil from entering the voids in the gravel surrounding the distribution trench. The building paper developed a vapor barrier which made it impossible to evaporate any water from the soil adsorption field. Now, untreated building paper, straw, or other synthetic drainage fabric materials are most often used which allow more evapotranspiration to take place. To my knowledge, in areas in the midwest, where rainfall just about equals evapotranspiration on a yearly basis, one does not credit evapotranspiration in the sizing of the soil absorption field even though it is advisable to encourage it in the system design.

Q: Morrison

I have another part to the question. What about covers or even the use of solar heaters to speed up evapotranspiration?

A: Boyle

There has been some research done at the University of Colorado using rotating discs to increase the surface exposure of the wastewater to the air; thereby, achieving very high rates of evaporation. I am not aware of the use of solar heaters, although I know that the use of greenhouses with adequate ventilation have been suggested to improve evapotranspiration. I would say that most of the successful evapotranspiration systems that are used in the U.S. are found in the West where the solar insolation is high and there is a favorable vapor pressure differential.

Q: Morrison

May I ask one more brief question? Do you know of any use of synthetic fills as a filtration barrier or adsorbent in any of these systems that are in place?

A: Boyle

To transmit the wastewater into the soil?

Q: Morrison

Right.

A: Boyle

No, I do not.

Q: Morrison

Thank you.

Q: Rohlich

Do you have any information, Bill, on the comparative costs of a pump system versus a gravity-flow septic tank?

A: Boyle

I don't have those numbers in mind. Do you know what they are, Jim?

Q: Kreissl

The numbers as far as economics?

A: Boyle

Cost comparison, just relative.

Q: Kreissl

We made a rough shot at it to the effect that it would increase the cost of the system depending on the soil. Anywhere from about 50 percent to 80 percent of the total system cost. That is the life-cycle costs including the amortized capital as well as operating. If I could comment, well I am responding really, to Dr. Morrison, we did do some research at the University of Colorado on covers. The trouble with covers is as you well know, the sizes of the systems are so huge that you really are into an economic situation. Already in Colorado I believe at least 5,000 ft^2 is required and it may be closer to 10,000 ft^2. Then you have to put a cover over that and you are talking about an awful lot of money.

A: Boyle

Relative to your question of cost of pressure distribution vs. gravity, Dr. Rohlich, I would say that operation and maintenance costs for this pressure distribution system would be very low.

Q: Rohlich

My second question, Bill, is to what extent has there been multiple port discharge of gravity flow? You talk about the problem of increasing carcinogens. Aren't there head boxes with multiple port discharge which can induce the flow at different points?

A: Boyle

There are distribution boxes which are employed on fairly level sites which are used to give multiple discharges to several laterals in the field. The problem with these systems is getting the outlet inverts level and having them stay that way. What very often happens is that one line drains everything and the others stay empty all the time. The design and construction of these systems is very critical and the quality control for small systems is such that very often they simply don't work very well. Distribution may also be provided by drop box or relief line networks for continuously ponded multi-trench systems on level or sloping terrain. Again, good design and construction are essential for proper performance.

Q: Rohlich

Well, I was talking about port discharges at different points and even single-lateral. In other words, introducing the flow let's say at four points.

A: Boyle

We've got perforations all along the pipe. It just goes through the point of least resistance. I am not familiar with the use of ports on an individual lateral.

Q: Rohlich

I guess I am talking about a manifold design.

A: Boyle

Yes, with the pressure distribution that's used all the time but with gravity distribution with the exception of the use of multiple ports in addition to using boxes I'm not aware of any.

Q: Salvato

Have you had any experience with drop manholes for the more equitable distribution of wastewater to the absorption field?

A: Boyle

The use of drop boxes?

Q: Salvato

Yes, a series of drop boxes say on a slope.

A: Boyle

Yes, they are more expensive but have a number of advantages over single line, closed loop, or distribution box networks. They are quite effective on steeply sloping sites, allow for resting of unneeded portions of the soil absorption field, and provide flexibility in the soil absorption field expansion.

Q: Audience

They are used in Wisconin but not extensively.

A: Boyle

Wisconsin is relatively flat.

Q: Salvato

I would like to point out in reference to evapotranspiration system design that a critical feature in achieving a satisfactory water balance is the percentage of the precipitation that runs off versus the percentage that infiltrates into the bed which would add to the load. With proper design and a favorable water balance the evapotranspiration system can function, particularly from my own experiences for summer operations. For year-round operations you have to have a very careful design considering where you live and surface runoff.

A: Boyle

Thank you.

Q: Davidson

I see a lot of emphasis placed on saturated hydraulic conductivity and yet one of the first things in a septic tank system we strive for is a mat which leads to an unsaturated condition. Thus, should we not be placing more emphasis on the development of the mat and the potential it has to produce unsaturated conditions? Also, perhaps we should be measuring unsaturated conductivities as a function of degree of saturation rather than saturated hydraulic conductivities.

A: Boyle

Exactly. We are really not concerned at all with the saturated hydraulic conductivity. It is the unsaturated hydraulic conductivity for a given moisture tension created by the mat that gives us a real insight into how a soil is going to behave under that mat resistance. We do not have very much data on the equilibrium mat resistances which result after a period of operation. It is that kind of information we need if we ever expect to rationally design these kinds of systems.

CONTROL MEASURES - LAND APPLICATION

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ABSTRACT

Control options available for the reduction of pathogens at wastewater land application facilities are described. The effectiveness of upgrading treatment facilities; improved operation and maintenance of existing facilities; and appropriate site selection, management and operation as control measures are discussed.

Most conventional wastewater treatment processes are not designed for pathogen removal, although some unit processes do impact the populations of microorganisms found in raw sewage. Only disinfection is included in the treatment train for the reduction of microorganisms discharged in the effluent.

The actual effectiveness of unit processes for the removal of pathogens from wastewater is not well understood. The overwhelming majority of information available on this topic has been developed using laboratory strains of organisms and model or laboratory-scale treatment units. These laboratory systems are not subjected to normal variations in hydraulic and organic loading and other variables found under field operating conditions. Furthermore, exogenously added test organisms may not respond to removal or inactivation as do indigenous organisms.

This paper stresses the need for information on the effectiveness of wastewater treatment unit processes in the removal of pathogens under a wide range of field operating conditions. Furthermore, the effect of normal variations in hydraulic and organic loadings on the reduction of microorganisms needs to be determined. When these data are available, a positive statement can be made regarding the control of microorganisms by conventional wastewater treatment processes.

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CONTROL MEASURES - LAND APPLICATION

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INTRODUCTION

The occurrence of bacterial, protozoan, helminthic and viral pathogens in domestic wastewater has been thoroughly documented. Table 1 provides a partial listing of these pathogens as well as their associated diseases. Concentrations of pathogens in raw domestic wastewater will vary from community to community and will be a function of the community's demography, its geographical location and the season of the year.

Much has been reported as to the transport and fate of these pathogens in soils and in groundwater. Furthermore, there has been considerable discussion as to the implications of discharging these pathogens to the land through the practice of land application of wastewaters.

If this Conference is to conclude that the presence of pathogens in applied wastewater represents a risk to public health, then it is only appropriate that this body made recommendations as to their control. More specifically, recommendations should be made for the reduction of that risk to an acceptable level. Thus, it is the intent of this paper to attempt to place in perspective the control options which may be available.

CONTROL OPTIONS

Traditional control options available for the reduction of contaminants in wastewater discharges include reduction of the contaminant at the source, upgrading treatment processes, and improved operation/maintenance of existing treatment facilities. An additional option exists at land application facilities; this involves appropriate site selection, management and operation.

With regard to the simplistic case of domestic wastewater, the source reduction option for pathogens is not practicable or appropriate for consideration. Thus, the remainder of this discussion will be limited to the other options identified above.

Treatment Processes

When selecting a wastewater treatment train appropriate for the reduction of pathogens it is important to understand the fate of pathogens within the various unit processes available to the designer. Conventional wastewater treatment facilities are designed to reduce the amount of organic material (as measured by the biochemical oxygen demand test) and suspended material

TABLE 1. Some Pathogens Found in Sewage and Their Associated Diseases

Organism	Disease
Bacteria	
Salmonella	Salmonellosis
Shigella	Bacillary dysentery
Mycobacterium	Tuberculosis
Enteropathogenic *Escherichia Coli.*	Gastroenteritis
Yersinia enterocolitica	Gastroenteritis
Protozoa	
Entamoeba histolytica	Amoebic dysentery
Giardia lamblia	Anorexia
Helminths	
Ascaris lumbricoides	Ascariasis
Ancylostoma duodenale	Anemia
Nector americanus	Anemia
Taenia saginata	Abdominal discomfort
Viruses	
Poliovirus	Paralysis; aseptic meningitis
Coxsackievirus	Aseptic meningitis, gastroenteritis
Echovirus	Aseptic meningitis
Reovirus, Rotavirus	Mild respiratory infection; gastroenteritis
Adenovirus	Acute respiratory infection
Hepetitis virus	Infectious hepatitis
Norwalk agent	Gastroenteritis

discharged to natural waters. Only the disinfection process is intentionally included for the reduction of concentrations of microorganisms discharged in the effluent. Nevertheless, some wastewater treatment unit processes do impact the populations of microorganisms found in raw sewage. Figure 1 provides a schematic of selected conventional wastewater treatment unit processes. Table 2 provides a summary of removal efficiencies for conventional wastewater treatment unit processes as reported by a number of investigators (1).

Unfortunately, the overwhelming majority of the information available on pathogen removal by treatment processes has been developed using laboratory strains of organisms and model or laboratory-scale treatment units. These laboratory systems are not subjected to normal variations in hydraulic and organic loading and other variables found under field conditions. Furthermore, exogenously added test organisms may not respond to removal/inactivation as do indigenous organisms. Equally important is the fact that results obtained from the evaluation of a few strains of microorganisms cannot be unequivocally extrapolated to all pathogens of concern. Consequently, much of the published data will represent an overestimate of pathogen removal efficiencies and, thus, must be interpreted with caution as to its reproducibility or applicability to field conditions.

Treatment process effectiveness can have a marked effect on the performance of pathogen detection methods. Furthermore, the actual wastewater treatment process train employed dictates to a large degree the disposition of the pathogens.

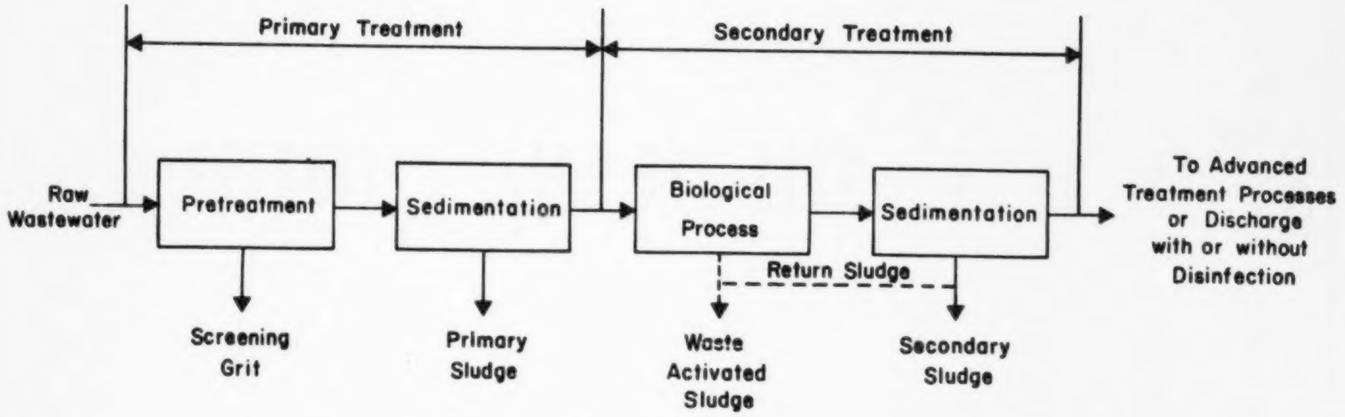
Primary Treatment

Most conventional wastewater treatment facilities consist of some preliminary processes such as pumping, screening, and grit removal. For all practical purposes these processes should not affect the levels of pathogens found in the wastewater.

The first major process in a conventional wastewater treatment facility is that of primary treatment. Primary treatment is sedimentation, and it is designed to remove heavy solids and floatable materials. Primary sedimentation basins with overflow rates of 600 gallons per day per square foot (gpd/ft^2) can remove 40-60 percent of the suspended solids and perhaps up to 35 percent of the 5-day biochemical oxygen demand (BOD_5) found in raw municipal wastewater.

Primary sedimentation has been shown to provide minimal virus removal. Malherbe and Strickland-Cholmley reported removals up to 69 percent for poliovirus(2). In studies involving polioviruses 1, 2 and 3 under field conditions, England and co-workers reported removals of between 0 to 12 percent(3).

Sproul, in his review of the effectiveness of conventional wastewater treatment processes on pathogen removal, indicated that primary sedimentation provided incomplete removal for all organisms and occasional increases in the numbers of viruses detected(1). The latter phenomenon has been attributed to fecal solids breaking up in the sedimentation tank thereby releasing viruses to the wastewater stream.


Primary Treatment
Secondary Treatment
Raw Wastewater
Pretreatment
Sedimentation
Biological Process
Sedimentation
To Advanced Treatment Processes or Discharge with or without Disinfection
Screening Grit
Primary Sludge
Return Sludge
Waste Activated Sludge
Secondary Sludge

Selected Biological Process Options

Trickling Filtration:

- Low Rate
- High Rate
- Two-Stage
- Biological Towers

Stabilization Ponds

Activated Sludge:

- High Rate
- Step Aeration
- Conventional
- Contact Stabilization
- Extended Aeration (without primary sedimentation)

Figure 1. Selected Conventional Wastewater Treatment Options

TABLE 2. Removal of Organisms by Conventional Wastewater Treatment(1)

Agent	Removal (%)	System
	PLAIN SEDIMENTATION	
Viruses:		
Poliovirus 1	0	Bench
	Incomplete to 69% removal	Plant
Poliovirus 1,2,3	0-12	Plant
Parasites:		
Beef tapeworm eggs	50	Bench
E. histolytica cysts	0 to incomplete removal	Plant
Bacteria:		
Tubercle bacilli	50	Plant
Coliform	27-96	Bench
	TRICKLING FILTERS	
Viruses:		
Coxsackievirus A9	94	Bench
Echovirus 12	83	Bench
Poliovirus 1	85	Bench
Mixed (natural)	Incomplete to 69	Plant
Parasites:		
Beef tapeworm eggs	30	Bench
E. histolytica cysts	Incomplete	Plant
	90-99.9	Bench
Bacteria:		
Tubercle bacilli	45	Plant
Salmonella typhi	72	Plant
Coliform	98	Plant
Pseudomonas aeruginosa	+74 (increase)	Plant
Clostridium perfringens	92	Plant
	ACTIVATED SLUDGE	
Viruses:		
Coxsackievirus A9	96-99	Bench
Poliovirus 1	79-94	Bench
Mixed (native)	53-71	Plant
Poliovirus 1,2,3	76-90	Plant
Parasites:		
Beef tapeworm eggs	0	Bench
E. histolytica cysts	Incomplete	Plant
Bacteria:		
S. typhi	86-99	Bench
Vibrio cholerae	96-100	Bench
Tubercle bacilli	90+	Bench
Coliform	97	Bench
Fecal streptococci	96	Bench

Secondary Treatment

Secondary wastewater treatment is a biological process involving aeration to metabolize and flocculate colloidal and dissolved organics. In addition to the biological processes (generally some form of activated sludge or trickling filtration), final clarification (or sedimentation) of the wastewater is accomplished. This part of the process separates the solids generated through biological metabolism from the liquid stream.

Satisfactorily operating secondary wastewater treatment processes will remove 90 percent or more of both the BOD_5 and suspended solids originally present in the raw wastewater. Generally, this will result in BOD_5 and suspended solids levels of less than 30 mg/L in the process effluent.

Activated sludge processes involve the suspension of microorganisms in wastewater. Air is added to provide an aerobic environment and to ensure adequate mixing. The aeration basin (reaction chamber) is the heart of the activated sludge system. Its design and operation is governed by a number of variables including organic loading, detention time, and the concentration of microorganisms in suspension. The ranges of general operating variables for selected types of activated sludge processes are shown in Table 3.

TABLE 3. General Operational Variables for Selected Types of Activated Sludge Processes

Type Process	Aeration Basin		MLSS+ in Aeration Basin (mg/L)
	Loading F/M*	Detention Time (hours)	
High Rate	0.4 - 1.5	0.5 - 2	4,000 - 10,000
Step Aeration	0.2 - 0.4	5 - 7	2,000 - 3,500
Conventional	0.2 - 0.4	4 - 8	1,500 - 3,000
Contact Stabilization			
Contact basin	0.2 - 0.6	0.5 - 1	1,000 - 3,000
Stabilization basin	---	3 - 6	4,000 - 10,000
Extended Aeration	0.05 - 0.15	18 - 36	3,000 - 6,000

*Pounds of BOD_5/day per pound of MLSS in the aeration basin
+Mixed liquor suspended solids

The variables shown in Table 3 clearly indicate that there is a wide range in operating conditions available for the different types of activated sludge processes. Furthermore, these are typical design ranges which are based on average daily BOD_5 in the influent to the process and average daily flow rates, conditions which exist for very short periods of time during any given day.

In evaluating the performance of activated sludge processes (or any treatment process, for that matter), it is important to describe the type of process involved, variations in flow and organic loading, and actual operating conditions when they deviate from the design assumptions. Flowcomposite sampling is essential to accommodate daily variations in flow and microbiological loading. Thus, laboratory (bench) studies rarely simulate conditions that exist at field sites.

The activated sludge process has been shown to be effective for the removal of large numbers of viruses from the liquid portion of wastewater. In one study Moore and co-workers evaluated a 10 million gallons per day (MGD) contact stabilization plant located in Austin, Texas (4). The plant operated with a contact time of 20 to 30 minutes followed by a four-hour sludge stabilization period. Using grab sampling techniques, indigenous enteric virus levels detected in raw wastewater entering the plant were shown to remain fairly constant at around 1,000 pfu/L with a decrease to only 250 pfu/L during a low flow period. Heavy rains resulted in what appeared to be a flush of the wastewater collection system with the enteric virus levels reaching 1,500 pfu/L as the plant flow increased to 15 MGD.

Virus levels in the effluent of the contact stabilization process ranged from a low of approximately 10 pfu/L to a high of 300 pfu/L. Sludge-associated virus levels consistently ranged from 5 to 10-fold greater than "free" virus reaching 900 pfu recovered from the solids of a one liter volume of mixed liquor. Under normal operating conditions, greater than 90 percent of the detectable incoming viruses were removed from the liquid fraction of the wastewater.

As indicated in Table 2 activated sludge processes have been shown to provide incomplete removal of E. histolytica cysts and the removal of 86 to 90 percent of the bacteria tested. However, it should be noted that data for bacteria were all derived from bench-scale studies.

Field studies encompassing a wide range of plants employing some form of activated sludge treatment have indicated that, generally, greater than 90 percent of the enteroviruses as well as the overwhelming amount of bacteria recovered in the aeration basin were solids-associated. Although these organisms are significantly reduced in the liquid fraction of the wastewater, they are concentrated in the sludges, a fact which must be considered in sludge disposal practices.

Trickling filtration is a process whereby biological growth is attached to a fixed medium. The medium may be natural (stone), ceramic, or synthetic (plastic). Sludge recycling is generally not required and the amount of sludge produced is far less than that produced by the activated sludge process. The quantity of biological growth developed is controlled by the available food (organic material), hydraulic loading to the medium (flow rate), the type of media, temperature, filter depth, and number of other factors.

As with activated sludge there exist a number of trickling filter systems with different design characteristics. Trickling filters are classified as to hydraulic loading, organic loading, depth, medium and recirculation. A partial listing of these classifications can be found in Figure 1.

Generally, trickling filtration is less effective in removing microorganisms than is activated sludge (see Table 2). Data compiled in a recent field study are provided in Table 4 and are used to illustrate this point with regard to viruses. In this case, the activated sludge process was an oxidation ditch. Both the oxidation ditch and the trickling filter were hydraulically underloaded and well operated. Flow composite sampling procedures were used in the study. The results are comparable due to the fact that the same raw wastewater served as the influent to both unit processes. Mean virus removal efficiency was 77.4 percent for the trickling filter as compared to 91.2 percent for the oxidation ditch. More dramatic differences were observed for bacteriophages while the differences for indicator bacteria were minimal (see Table 5).

Sproul noted that several studies have shown virus removals in trickling filters of up to 85 percent, removal of parasites to be poor and removal of bacteria to range from 0-98 percent (1). The increase in virus concentrations occasionally observed in trickling filter effluents is said to result from the breakup of fecal solids in the filter, the low biomass available for viral adsorption, and the relatively short contact time between the wastewater and the biological growth on the filter medium.

Advanced Wastewater Treatment

Advanced wastewater treatment involves the use of processes that remove more contaminants than are usually taken out by conventional wastewater treatment processes. More often than not the goal is to remove refractory compounds. Many of these compounds can adversely affect aquatic life in streams or lakes and preclude the reuse of the liquid stream for higher purposes.

There are a number of unit processes which are popular for advanced wastewater treatment. Included are chemical coagulation with sedimentation, granular filtration, and microstraining for suspended solids removal; adsorption on activated carbon or extended biological oxidation for organics removal; chemical coagulation and bio-chemical precipitation and clafification for phosphorous removal; and nitrification-denitrification, ammonia-air stripping, breakpoint chlorination and ion exchange for nitrogen removal. Membrane processes (reverse osmosis and ultrafiltration) have been shown to be highly effective in the removal of most refractory compounds, also.

From a practical point of view, however, there would be little or no economic justification for the installation of these processes at wastewater land application facilities.

Disinfection

It is generally agreed that despite the effectiveness of a single or even most multiple unit processes for the removal of microorganisms from wastewater, the level of reduction achieved may not adequately provide the margin of safety required for many uses or discharges of wastewater. The major process employed to insure low microorganism concentrations in treated wastewater is disinfection. In the United States disinfection is practically synonymous with chlorination for most applications.

TABLE 5. Effectiveness of Two Biological Processes in Reducing Indicator Bacteria and Bacteriophages (5)

	Mean Concentration* and Removal (%)		
	Total Coliform (cfu/100 ml)	Fecal Coliform (cfu/100 ml)	Bacteriophage (pfu/L)
Raw Wastewater	1.7×10^{8}	4.4×10^{7}	8.6×10^{6}
Oxidation Ditch Effluent	9.6×10^{6}	2.4×10^{6}	7.7×10^{4}
(% Reduction)	(89%)	(95%)	(99.1%)
Trickling Filter Effluent	1.3×10^{7}	2.5×10^{6}	1.3×10^{6}
(% Reduction)	(92%)	(95%)	(15%)

*Based on sixteen 24-hour composite samples.

TABLE 4. Effectiveness of Two Biological Processes in Reducing Enteroviruses (Selected Sampling Days), Kerrville, Texas (5)

Sampling Point	Enterovirus Recovered (pfu/L, 5 day) and Treatment Effectivess (%) Sample Number 1	2	3	4	5	6	7	8	9	10
Raw Wastewater	9.1	6.8	31	8.0	7.0	5.6	70	780	910	34
Oxidation Ditch Effluent	0.7	0.3	2.0	1.3	0.5	2.2	1.8	1.0	5.7	1.2
(% Reduction)	(92%)	(96%)	(94%)	(84%)	(93%)	(61%)	(97%)	(99.9%)	(99.4%)	(96%)
Trickling Filter Effluent	2.5	0.7	1.6	1.1	2.1	1.9	21	320	140	7.0
(% Reduction)	(73%)	(90%)	(95%)	(86%)	(71%)	(66%)	(70%)	(59%)	(85%)	(79%)
Flow (% of Mean Flow)	105	102	99	94	109	84	103	105	105	97

A number of studies have demonstrated the variability in resistance to chlorine by different microorganisms. Generally, hypochlorous acid (HOCl) is the major chlorine species that occurs at lower pH and has been found to be a more effective bactericide and virucide than the major chlorine species found at high pH (hypochlorite ion). Further, combined chlorine species have been found to be less effective than either of the dominant free chlorine species (HOCl and OCl^-).

Most experimental work involving the inactivation of microorganisms with chlorine has been conducted in clean systems (essentially no suspended material) with "free" organisms added to the test system. Under these conditions the inactivation of the microorganisms is reasonably fast when free chlorine residuals are present.

The more extensive data from which Table 6 was extracted permit a comparison of survival rates of different viruses in river water containing 0.5 mg/L free chlorine residual. E. histolytica cysts require high chlorine residuals for their inactivation. Additional data provided in Table 7 suggest that bacteria are more susceptible to chlorine than are viruses and parasites.

Recent field studies have shown significant differences in the effectiveness of chlorine inactivation of indigenous fecal coliform and bacteriophage (7). Specifically, at a chlorine dose of 5 mg/l at pH 7.4-7.8, temperatures of 22-30°C, and a contact time of three minutes, a 2.5 $\log_{10}$ loss was observed for fecal coliform and a 0.8 $\log_{10}$ loss for coliphage. Similarly, at a chlorine contact time of 30 minutes, a 4.0 $\log_{10}$ loss was observed for fecal coliform while a 1.2 $\log_{10}$ loss was observed for coliphage.

TABLE 6. Time Required for 99.99 Percent Inactivation of Selected Human Viruses with 0.5 mg/L Free Chlorine *(6)

Virus	Time (minutes)
Reovirus 1	2.7
Coxsackievirus B2	6.5
Coxsackievirus A9	6.8
Echovirus 7	7.1
Adenovirus 7a	12.5
Echovirus 8	13.0
Poliovirus 1	16.2
Adenovirus 12	23.5
Echovirus 1	27.0
Poliovirus 3	30.0
Coxsackievirus B3	35.0
Poliovirus 2	40.0
Echovirus 12	> 60.0
Coxsackievirus A6	> 120.0

*In samples of Potomac River water at 2°C and pH 7.8

Unfortunately, none of the chlorine inactivation studies have included the waterborne virus of most concern, hepatitis virus, due to laboratory limitations in handling this virus. Further, there has developed an increasing concern that chlorine disinfection (or any disinfection, for that matter) may not be as effective for naturally occurring microorganisms as it is with laboratory-cultivated microorganisms. Data supporting this contention result from studies which consider the fact that a large part of the naturally occurring microorganisms are solids-associated and that solids are protective of microorganisms during disinfection.

In any case, chlorination will be most effective when the wastewater is relatively free of turbidity, the chlorine is mixed effectively so that the active species comes in contact with the viruses, and a free chlorine residual is insured for at least 30 minutes of contact time.

TABLE 7. Microorganism Inactivation with Chlorine
[After Sproul (1)]

Organisms	Condition	Inactivation (%)
Viruses:		
Poliovirus 1	0.5 mg/1 FAC* for 16 min; 2°C; pH 7.8	99.99
Polivirus 1	1 mg/L FAC for 2 min; 5°C; pH 6	99
Coxsackievirus A9	1 mg/L FAC for 2 min; 5°C; pH 6	99
Parasites:		
E. histolytica cysts	12 mg/L NH_2Cl, for 10 min; pH 9	99.9
E. histolytica cysts	3.2 mg/L for 30 min; 10°C; pH 7.0	100
Bacteria:		
Coliform	1 mg/L FAC for 0.25 min; 0-6°C	99
Salmonella typhi	0.1-0.3 mg/L FAC for 1 min; 20-25°C; pH 7.0	100
Shigella dysenteriae	0.07-0.1 mg/L FAC for 1 min; 20-25°C; pH 7.0	100

*Free available chlorine

Operation and Maintenance

An often overlooked aspect of wastewater treatment is daily operation of the in-place facilities. To derive the optimal benefits of any unit process requires proper operation and maintenance. With the advent of federal construction grants programs, many municipalities have installed relatively sophisticated wastewater treatment facilities. Operational costs are high and wastewater treatment often suffers in municipalities which have economic contraints.

A recent policy statement of the Water Pollution Control Federation makes this point quite emphatically (8). The statement indicates that only 50 percent of the public wastewater treatment facilities in the United States are performing properly. Among the reasons cited for this poor performance are inadequate manpower, inept or untrained operators, as well as inadequate supplies, budgets, and parts inventories. It seems that many communities have neglected to plan for the resources required to adequately operate those facilities they were so eager to construct, particularly with federal grant programs providing 75 percent of the construction costs. Unfortunately, there is no federal grant program for the operation and maintenance of wastewater treatment facilities. Similarly, optimal operation of many unit processes is not possible in older communities with combined sewers or sewer systems with high infiltration rates. During periods of heavy or prolonged precipitation hydraulic induced upset of the treatment processes can occur, thereby greatly reducing treatment efficiency.

Site Selection

Proper site selection and management of land application facilities is important to the performance of the system with regard to control of microorganisms. Criteria for site selection and the design of these systems have been described in EPA's Process Design Manual for Land Treatment of Municipal Wastewater (9) and have been summarized by Harlin (10).

As shown in Table 8 the mode of wastewater application selected will dictate the extent to which microorganisms could impact groundwater. Microorganism transport to groundwater is enhanced under a number of site conditions, including high soil permeability, coarse soils, low cation exchange capacity, high application rates and dramatic changes in the ionic nature of the percolating water. Clearly, if there is concern regarding the impact of microorganisms on local groundwaters, then the rapid infiltration approach should not be considered for that location. By the same token, care should be taken in the selection of sites for slow rate systems.

That the wastewater application system is performing as designed can only be determined through the institution of a monitoring program. The extent of this program will be dictated by the type of treatment provided prior to land application, the hydrologic nature of the site, and the degree of concern placed on the groundwater as a resource in the area. At present, it is a national policy that groundwater should not be degraded as a result of land application practices (11). Furthermore, monitoring land application systems should not be considered any different than monitoring wastewater treatment plant effluents which are discharged directly into receiving watercourses.

Caution must be excercised with this type monitoring program. For example, the use of ceramic lysimeters is of no value for determining microorganism concentrations of percolating waters as they do not efficiently penetrate the lysimeter. Adequate technology for monitoring viruses in groundwater has only recently been successfully demonstrated and extremely large volumes of water are required (as is the case of monitoring treated potable water). In addition, no existing single viral monitoring procedure is capable of recovering even a minor fraction of the total potential number of viruses present in water.

TABLE 8. Selected Characteristics of Land Application Systems

Factor	System		
	Overland Flow	Rapid Infiltration	Slow Rate
Application rate (ft/yr)	10-70	20-560	2-20
Land required for 1 mgd flow (acres, net)	16-110	2-56	56-560
Desired soil permeability	Low	High	Moderate
Probability of influence on groundwater	Slight	Certain	Probable
Evaporation	Moderate	Low	High
Treatment effectiveness	Moderate	Low	High

CONCLUSIONS

There is little question as to the capability of the various options discussed to be effective in the control of microorganisms discharged to land. The primary question centers around the extent of control afforded by these options.

Designers and operators often ask for specific information regarding the reduction of pathogens by a specific treatment process. More often than not, the response is based on coliform data or data developed in clean test systems or bench-scale systems.

Current knowledge points to the fallacy in such a response. We do know that there are differences between clean-beaker results and field observations. Furthermore, we know that there will be differences in the results observed in bench-scale studies when compared to adequately designed field studies. We also know that "normal" operating parameters at full-scale facilities are quite variable and will depend on a number of factors including adequacy of operation and maintenance, environmental conditions (i.e. temperature, precipitation, etc.), and unexpected discharges to the treatment system (i.e. industrial wastes). What we do not know is the extent of these differences.

To develop this type of information will require a significant effort. First, it must be demonstrated that methodologies capable of being both quantitative and sufficiently sensitive are as useful in the field as they are in the laboratory. Second, these methods must be used over a wide range of field operating conditions to ascertain the effectiveness of a number of unit processes for the reduction of indigenous pathogens. Furthermore, the effect of normal variations in hydraulic and organic loadings on the reduction of microorganisms needs to be determined. Only when these data are compiled and interpreted can a positive statement be made regarding the control of microorganisms by conventional wastewater treatment processes.

Thus, if this Conference is to conclude that there is a risk to human health from pathogens in wastewater applied to land, then it would seem logical and appropriate that it recognize the limits of our ability to predict the control of that risk by conventional treatment processes. Following that the conferees should then be prepared to recommend investigation of the problem so that answers to the questions can be developed.

In addition, we must recognize that there is little economic or practical value in attempting to reduce that risk to zero. What should be sought is a control strategy necessary to reduce the risk to a level which is acceptable by society. To quote Lowrance (12), ". . we must adjust our heading so as to enjoy the greatest benefit at the lowest risk and cost. . . "

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CONTROL MEASURES - LAND APPLICATION: CHARLES SORBER

TRANSCRIPT OF QUESTIONS/RESPONSES/COMMENTS

Q: Gerba

I would like to make two points in bringing the literature up to date on enteric viruses removed by sewage treatment processes. The review which you quoted was done some time ago and there is more recent literature which indicates that enteroviruses may be one of the easier groups of viruses to remove by conventional sewage treatment. Recent literature has appeared to indicate adenoviruses, reoviruses and work done in our own laboratory on rotaviruses which indicates that these viruses are far less effectively removed than enteroviruses by conventional sewage treatment processes.

A: Sorber

Yes, I agree but I thought I had made my point more clearly. What I am more concerned about is looking at removal efficiencies from an actual operating condition point of view and not specific virus strains under controlled laboratory conditions or with just grab sampling. What you are talking about is comparative removal efficiencies under specific conditions of a laboratory test.

Q: Morrison

I have a question that may not be directed toward any one person, but I would like to know from anyone here what their thoughts might be on what we need to monitor in the ground water at slow rate irrigation land treatment sites. Maybe fecal coliforms aren't what we need to monitor. Of course, we need to monitor nutrients, inorganics and organics; but my question is put in the framework of the typical small town that is not sponsoring a research project and has limited budget. What do we need to look for to protect the local people?

A: Sorber

Well, I will attempt to answer your question. I think that's what the panel is going to work on tomorrow. First, you have got to decide what the problem is, if there is a problem. Once that is decided, then one can identify the microorganisms which present that problem. If you make that judgment then you make a judgment as to what you look for. Now obviously the typical small town is not going to monitor for viruses. It is just not practical. So, the matter is then a function of selecting that indicator or group of indicator systems which would best permit a monitoring program to be installed, if such a program is considered necessary. But perhaps you don't need to monitor the ground water at all. Maybe what you need to monitor is what you are discharging to the land. It is a lot easier to monitor a concentrated stream than it is to monitor ground water. Thus, if you have a handle on the fate and transport of microorganisms and if you know what you are discharging to the land, then ground water monitoring might be unnecessary.

Q: __________

Would you recommend disinfection prior to land application?

A: Sorber

I don't recommend disinfection or anything else until I see the site and the situation that exists around the site. EPA's policy is to indicate minimum treatment as illustrated on Dr. Harlin's slide yesterday. As he pointed out then and as I tried to point out today, you upgrade that treatment, to include disinfection, as dictated by the needs of the particular site. It may seem to be a terrible cop-out to say that the answer to your question is "site-specific", but it is so very site-specific it is unreal. I don't think disinfection for the sake of disinfection is important or appropriate in the land application of wastewater.

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